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The epidemiology of brucellosis in animals and humans in Arusha and Manyara regions of Tanzania

by

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Abstract

The aims of this study were to assess community knowledge, awareness and practices on zoonoses, to gather baseline data on brucellosis in livestock and wildlife, to establish brucellosis seroprevalence in domestic ruminants and humans and risk factors associated with livestock seropositivity, to assess brucellosis dynamics and impact on livestock production and reproduction and to evaluate the performance of the Rose Bengal Plate Test (RBPT) in Tanzania. The results described in this study were carried out through Participatory Rural Appraisal (PRA), cross-sectional and longitudinal studies.

In the PRA and cross-sectional studies, rabies, tuberculosis, anthrax and brucellosis were the zoonoses most frequently identified. Cattle were frequently identified as being associated with tuberculosis, anthrax and brucellosis, whereas dogs were frequently identified as being associated with rabies. Small ruminants, pigs, cats and poultry were either infrequently, or not identified as being associated with zoonoses. Recognition of clinical signs of zoonoses in humans was better than in animals. Ingestion of animal products was a route frequently identified as transmitting zoonoses to humans.

During the baseline serosurvey, seroprevalences for brucellosis were 6.2% in cattle, 6.5% in small ruminants and 13% in wildlife, respectively. Seropositivity was significantly higher in the pastoral (13.2%), followed by agro-pastoral (5.3%), and lowest in the small holder dairy system (2.3%) ($p<0.05$).

During the cross-sectional serosurvey, the seroprevalence was significantly higher in older animals and large herds ($p<0.001$). Variation in seropositivity between households was

higher (1-30%) in the pastoral compared to agro-pastoral (1-14%) households. The model that best explained c-ELISA seropositivity included the feeding of dogs with foetuses and placentae, calving during the wet season, and the farming system.

In humans, 28% of families were seropositive for brucellosis with the highest levels in Ngorongoro district (46%), and lowest in Babati district (0%). Families with seropositive herds were 3.3 times more likely to be seropositive. However, 25% of families were seronegative when their herds were seropositive, and 48% of families were seropositive with seronegative herds.

In the longitudinal study, the incidence was 732/1,000 cases per animal-years at risk with an estimated survival probability of 0.836. Households with a high seroprevalence at the initial sampling had a high incidence of seroconversion in the subsequent visits. Occurrence of new seropositive cases was significantly higher in the wet season ($p< 0.05$). Calf serostatus was statistically associated with dam serostatus but no significant difference in growth rate was observed between calves suckled from seropositive and seronegative dams.

Brucella melitensis type-1 was isolated from goats' milk following culture. Blood and placenta samples were negative on bacteriological culture. The RBPT was found to have low sensitivity in both field and laboratory settings.

Brucellosis infection in livestock is widespread, but poses the greatest risk to human health and livestock production in pastoral systems in Tanzania.

Declaration

The research work reported in this thesis is my own original work, unless stated otherwise and has not been submitted elsewhere for any degree award.

Sign.....

Date:30 May 2005

Dedication

This thesis is dedicated to my beloved father Francis Mkilema Swai Makara who passed away June, 2004 while I'm writing this thesis at UK. It is also dedicated to my wife, Theresia Mamkenda and children; Francis, Elizabeth, Gloria, Emaculata, Hildergalda and Kona. I'm grateful to them for their patient, moral support and understanding that contributed to my success. Also dedication is extended to my beloved mother Elizabeth Mkashabani moisso and the late Mwal. Regina Edward for make sure I attended school properly.

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Abbreviations and acronyms

^0C	Centigrade
ANOVA	Analysis of Variance
AR	Attributable Risk
c-ELISA	Competitive Enzyme Linked Immuno-Sorbent Assay
CFT	Complement Fixation Test
CI	Confidence Interval
Cm	Centimetre
DF	Degree of freedom
DFID	Department For International Development
ECF	East Coast Fever
FAO	Food and Agricultural Organisation
FMD	Foot and Mouth Disease
FPA	Fluorescence Polarisation Assay
FRBPT	Field Rose Bengal Plate Test
GDP	Gross Domestic Product
MAC	Ministry of Agriculture and Co-operatives
OIE	The Office International de Epizooties
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PRA	Participatory Rural Appraisal
SARS	Severe Acute Respiratory Syndrome
SAT	Serum Agglutination Test
SUA	Sokoine University of Agriculture
Tsh	Tanzanian shilling
UK	United Kingdom
VIC	Veterinary Investigation Centre
VLA	Veterinary Laboratory Agency
WHO	World Health Organisation
K	Kappa

χ^2	Chi-square statistic
LRBPT	Laboratory Rose Bengal Plate Test
RBPT	Rose Bengal Plate Test
S-LPS	Smooth Lipo-polysaccharides
R-LPS	Rough Lipo-polysaccharides

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background information

Brucellosis, caused by a variety of *Brucella* species, is a disease of major socio-economic importance in domestic animals worldwide; especially so in developing countries where disease control programmes are either non-existent or inadequate. The disease also occurs in wild animals, thus posing a danger of transmission between domestic and wild animals in interface areas. *Brucella* species that cause disease in domestic and wildlife include *B. abortus, B. melitensis, B. ovis, B. suis* and *B. canis* (Corbel, 1988; Abdel-Hafeez *et al.*, 1995; Alonso-Urmeneta *et al.,* 1998). *Brucella abortus, B. melitensis, B. suis* and *B. canis* can all cause human brucellosis, whereas *Brucella ovis* and *B. neotomae* have not been reported to cause disease in humans. The disease is economically important as it is associated with abortion storms in newly infected herds, a high level of retained placentae and hence endometritis or metritis resulting in reduced milk production, infertility (Radostits, *et al.,* 2000) and high costs of treatment of human infections.

Animals become infected by ingesting contaminated pastures, feedstuffs and water or licking infected placentae, foeti or the genitalia of infected female animals soon after abortion or delivery (Corbel, 1988; Bishop *et al*,. 1994). Colostrum and milk from infected dams is also a potential source of infection for newborns (Bishop *et al.*, 1994). Venereal

transmission in domestic ruminants is uncommon except in areas where artificial insemination is used.

The disease was once a major problem in developed countries and was controlled through strict and scrupulous control regimens including improved hygiene, test and slaughter policy, vaccination and monitoring of animal movements (Meldrum, 1995; 0'Neal, 1996; Corbel, 1997). Brucellosis, however, has remained endemic in wildlife populations in some developed countries. For instance, *Brucella* antibodies have been reported in wild boar (*Sus scrofa*), American bison (*Bison bison*) and reindeer (*Rangifer tarandus subspecies platyrhynchus*) in France, Italy, Canada and Switzerland (OIE, 2000).

In developing countries, brucellosis continues to be a major problem in livestock and is known to occur in a number of African countries, in the Mediterranean region, South America and Eastern Asia (WHO, 1997). Brucellosis in wild animals has been reported in several African countries such as Zimbabwe (Madsen and Anderson, 1995), Tanzania and Kenya (Waghela and Karstad, 1986), and South Africa (Bishop, *et al.,* 1994).

In Tanzania, brucellosis was first confirmed in livestock in 1928 from samples taken from aborted cattle at Engare Nanyuki in Arusha region (Mahlau and Hammond, 1962; Kitaly, 1984). Since then, several surveys have shown the presence of the disease in livestock with variable seroprevalence (Table 1.1).

Table 1.1: The seroprevalence of animal brucellosis in different regions of Tanzania

Regions	*Sampling method*	*Test used*	*Seroprevalence (%)*	*Reference*
Morogoro	Convenient and purposive	Indirect ELISA	2-90	Minga and Balemba, 1990
Morogoro	Not known	SAT	5.6	Swai, 1997
		RBPT	10.6	
		Indirect ELISA	22	
Dar-es-Salaam	Purposive	SAT	14.1	Weinhaupl, *et al.*, 2000
Coastal	Convenient	SAT	12.3	
Dodoma	Purposive	SAT	5.2	Kitalyi, 1984
Arusha	Not known	SAT	6.9	Staak and Protz, 1973
Arusha	Multistage random sampling	IELISA	3.2	Minja, 2002
Mwanza	Purposive	SAT	10.8	Jiwa *et al.*, 1996
Mtwara/Lindi/Ruvuma	Not known	SAT	2-13	Otaru, 1985

Human brucellosis commonly referred to as "Undulant fever" or "Malta fever" is an important zoonosis that often coincides with livestock infection (Thimm and Wundt, 1976). Although most *Brucella* species are known to cause the disease in humans, *B. melitensis* is considered to be the most pathogenic (WHO, 1997). The disease in humans is acquired through ingestion of animal products and by contact with infected materials and the transmission rate from animals to humans can be influenced by endemicity of animal infection, farming systems, food consumption habits, hygienic standards and socio-

economic standards (Alausa, 1980; Abram, 1985; Dessai *et al.*, 1995; Johns, 1996; Schussler, *et al.*, 1997; WHO, 1997; Radostits, *et al.*, 2000). Controlling the disease in livestock has often resulted in a significant reduction in human brucellosis in developed countries. In areas where the disease is still endemic in livestock, such as those in the developing world, human infection is not uncommon (WHO, 1997).

1.2 Historical perspective and aetiology

1.2.1 Historical perspective

Brucellosis was first suspected to occur in humans presenting with symptoms such as malaise, anorexia, fever and profound muscular weakness. This was reported by Marston in 1861 and, as such, the condition was called "gastric remittent fever" (Joklik, *et al.*, 1980). The causative agent was isolated from the spleen of patients by a British scientist, Sir David Bruce in 1887, who named it *Micrococcus melitensis*. The genus *Micrococcus* was derived from its morphology and the species name from "Melita", the Roman name for the Isle of Malta where the disease was first recognised. Based on the description of the clinical illness, Hughes changed the name from "gastric remittent fever" to "undulant fever" in 1897 (Joklik, *et al.*, 1980).

A Danish veterinarian called Bang isolated *Brucella abortus,* originally called Abortus Bacillus of Bang from aborted cows in Denmark in 1897. He linked the organism to infectious abortion in animals. Due to the close bacteriological and serological relationship between *M. melitensis* and Abortus Bacillus of Bang, Alice Evans changed the genus and named it *Brucella* in honour of Sir David Bruce. The third member of the genus was

isolated by Traum from premature piglets following abortion in 1914 and it was called *Brucella suis*. The fourth member of the genus was isolated from sheep by Buddle and Simmons in Australia and New Zealand in 1953 (Topley and Wilson, 1990) and was named *Brucella ovis*. In 1957, Stoenner and Lackman isolated another *Brucella* organism from desert wood rats in USA and called it *Brucella neotomae*. *Brucella canis* was reported in the USA by Carmichael and Brunner in 1968 following isolation from dogs. To date six species have been described and accepted officially. In 1994 however, another *Brucella* organism was isolated from marine mammals and is unofficially designated as *Brucella maris* (Aleixo, *et al.*, 1999).

1.2.2 Aetiology

Brucella organisms are small, fastidious, non-motile, non-spore forming and facultative intracellular bacteria. They are either coccobacilli or short bacilli with a size range of 0.5-0.7μm wide by 0.6-1.5μm long (Joklik, *et al.*, 1980). They can occur singly, in groups, or in chains, and grow well on media containing blood or serum (Topley and Wilson, 1990). *Brucella* organisms are gram negative but often resist decolourisation following counterstaining. Biochemically, *Brucella* organisms oxidise certain amino acids such as L-glutamic acid and L-asparagine and certain carbohydrates such as D-glucose and I-erythritol (Toppley and Wilson, 1990). *Brucella abortus, B. melitensis, B. suis* and *B. neotomae* may occur as either smooth or rough strains expressing smooth-lipopolysaccharide (S-LPS) or rough-lipopolysaccharide (R-LPS) as major surface antigens, while *B. ovis* and *B. canis* are naturally rough strains.

1.3 Epidemiology of brucellosis in animals and humans

1.3.1 Distribution and prevalence

1.3.1.1 Livestock

Brucellosis occurs in most parts of the world (Chukwu, 1985; Corbel, 1997). It was once an important disease in developed countries but has been eradicated in several countries through test and slaughter, vaccination and restriction of animal movements (Meldrum, 1995; O'Neal, 1996). Although the incidence of brucellosis has been reduced to a low level or eradicated in developed countries, in other parts of the world such as the Mediterranean region, the Middle East, Western Asia, and parts of Africa and Latin America, its magnitude has increased due to increased animal production, intensive keeping of animals under poor hygienic conditions, in addition to social-economic and behavioural factors (Abdussalam and Fein, 1976). In many of these areas, the prevalence of animal brucellosis is high (Lulu *et al.*1988; Amato, 1995).

Brucellosis in livestock is known to occur in a number of African countries, albeit with varying prevalence rates (Thimm and Wundt, 1976). Studies have reported the prevalence to be 2% in Sudan (Mahmoud, 1991), 4% in Ethiopia (Tekelye *et al.* 1989), 6-18% in Kenya (Waghela, 1977; Ndarathi and Waghela, 1991), 10% in Somalia (Wernery *et al.* 1979), 7- 50% in Nigeria (Eze, 1977), 38-62% in Egypt (Refai *et al.* 1990), 18% in Uganda (Ndyabahinduka and Chu, 1984), 23% in Mali (Tounkana *et al.*, 1994), 8% in Burkina Faso (Coulibaly and Yameogo, 2000), 9% in Ghana (Turkson and Boadu 1992)

and 2-97%) in Tanzania (Mahlau, 1967; Kitaly, 1984; Otaru, 1985; Minga and Balemba, 1990; Swai, 1997; Niwael, 2001; Minja, 2002). These data are difficult to compare due to variation in sampling techniques and serological tests used.

The history of brucellosis in Tanzania dates back to 1927 when an outbreak of abortion was reported in Arusha region (Kitaly, 1984). The first laboratory confirmation of brucellosis was performed in 1928 from three aborting cattle from Engare Nanyuki in Arusha region (Anon, 1928). Since then, a number of studies have been carried out to establish the prevalence of disease in the livestock sector. Surveys have shown the disease to occur in cattle in various regions and zones, with seroprevalence varying considerably. The results of those surveys include 15% in Mwanza (Mahlau and Hammond, 1962), 15.2% in Arusha (Mahlau, 1967), 3.3% and 7.6% in Mbulu and Masailands (Staak and Protz, 1973), 5.2% in Central zone (Kitaly, 1984), 10.8% in the Lake zone (Jiwa et al., 1996), 12-14% in Eastern zone (Swai, 1997, Weinhaupl *et al.*, 2000), 3.2% – 4.2% in Manyara region (Niwael, 2001; Minja, 2002) and 15.2% in Southern zone (Otaru, 1985). Mahlau, (1967) went further by isolating *B. melitensis* from aborting goats and *B. abortus* in aborting cows in Iringa and Arusha regions, respectively.

1.3.1.2 Wildlife animals

Brucellosis remains a problem among several wild animal species in developed and developing countries. In the developed world, wildlife brucellosis has been reported to occur in the Yellowstone National Park in the USA (Cheville, *et al.,* 1998). Studies in the park showed that 50% of 1079 American bison *(Bison bison)* tested were seropositive to *Brucella* antibodies (OIE, 1997). *Brucella* organisms were also isolated from American bison, reindeer *(Rangifer tarandus subspecies platyrhynchus)* and caribous (*Rangifer tarandus subspecies caribou*) in Canada (OIE, 2000). In Europe, *Brucella* organisms were isolated from wild boars *(Sus scrofa)* in France and Italy, and brown hares *(Lepus timidus)* in Austria and Switzerland (OIE, 2000). Isolation of *Brucella* species has also been reported in marine mammals in Europe and the USA (OIE, 2000). Aquatic mammals known to be affected by the disease include beluga whales *(Delphinapterus leucas)* and ringed seals *(Phoca hispida)* (OIE, 2000).

Several developing countries, including African countries, have reported the infection in wild animals. Wildlife surveys conducted in Zimbabwe revealed a seroprevalence of 6.5%, 1.4%, 0.9% and 0.05% in buffalo, eland antelope, giraffe and impala, respectively (Madsen and Anderson, 1995). In addition, Bishop *et al.*, (1994) reported African buffalo, hippotamus, zebra, eland and impala to be serologically positive to *Brucella* antibodies in the Kruger national park in South Africa. In Tanzania, *Brucella* infection was reported in topi, buffalo, impala, Thomson gazelle and wildebeest (Sachs, *et al.,* 1968; Schiemann and Staak, 1971).

1.3.1.3 Humans

Although human brucellosis is a notifiable disease in many countries, official figures do not reflect the actual number of people infected each year. Thus, the true incidence has been estimated to be 10-25 times higher than that reflected in existing reports (WHO, 1997). This discrepancy could be attributed to infections remaining unrecognised because of inaccurate diagnosis or diagnoses of "pyrexia of unknown origin". Therefore, human brucellosis remains a public health burden in many developing countries (Ndyabahinduka and Chu, 1984), and its incidence in endemic areas varies from 1 to 200 per 100,000 people (Lopez-Merino, 1989). For instance, in Mediterranean and Middle Eastern countries, the annual incidence of human brucellosis was reported to vary from 1 to 78 cases per 100,000 people (Corbel, 1997). The prevalence in some African countries is very variable ranging from 5 to 55% in countries such as Nigeria (Alausa and Awosey, 1976), Benin (Fayomi, *et al.,* 1987), Burundi (Laroche, *et al.,* 1987) and Uganda (Ndyabahinduka and Chu, 1984).

In Tanzania, very little is known about human brucellosis. The first report of the disease was in 1935 (Anon, 1935). Further reports of human brucellosis in the country were from the Medical Department of the Lake and Western Regions in 1959, 1960 and 1961 where three cases were confirmed (Anon, 1962). Minja (2002) conducted a random survey in Arusha in different occupational groups and found a seroprevalence of 0.7%. Hospital reports indicate that, of 2013 *Brucella* suspect cases examined in 1999, 13% were seropositive for *Brucella* antibodies (Shirima, 2000 unpublished). The majority of these studies, however, did not isolate the organism that would have established which species is affecting humans in Tanzania.

1.3.2 Transmission

1.3.2.1 Livestock

Brucellosis occurs in animals of all age groups, but persists commonly in sexually mature animals (Adams, 1998). Infection is frequently introduced into clean herds or flocks through the introduction of infected animals which are either pregnant, that have recently delivered, or aborted. Transmission among animals is mainly through ingestion of contaminated pasture, water and feeds. Furthermore, licking infected placenta, young stock, foeti, or the genitalia of infected animals soon after abortion or normal delivery could also predispose animals to infection (Corbel, 1988; Bishop *et al.*, 1994). Milk and colostrum from infected animals are important sources of infection for young stock. It has been demonstrated that young stock born from positive dams can persistently harbour the infection and may be seronegative until abortion or normal delivery occurs (Cattlin and Sheehan, 1986). Transmission of *Brucella* organisms through inhalation, and via the conjunctiva, has also been reported (Bishop *et al.*, 1994).

Corbel (1988) demonstrated that although male animals can be infected in early life and retain infection for life, they are rarely responsible for the introduction or spread of infection to female animals by natural service. Transmission occurs when semen of infected bulls is used in artificial insemination (Corbel, 1988). Therefore, in areas where artificial insemination is uncommon as in the pastoral and agro-pastoral farming systems in Tanzania, males probably have only a minor role in disease transmission. However, semen used for artificial insemination is usually collected from brucellosis free bulls. Radostits

and colleagues (2000), suggested that such bulls should be serologically and bacteriologically negative.

Studies have shown that contaminated materials from other domestic animals can also be potential sources of infection. For instance, *Brucella melitensis* has been recovered from droppings, egg yolk, egg shell and internal organs of experimentally infected chickens (Abdullah *et al.*, 1984). Authors suggested the significance of chickens in the dissemination of brucellosis to man and domestic animals (Kudi *et al.*, 1997). Forbes (1990), also isolated *B. abortus* from dogs on *Brucella* infected farms and these may play an important role in disease dissemination, especially where close contact and environmental contamination are high (Wang *et al.*, 1995). Other factors that have been observed to influence the risk of *Brucella* infection include husbandry practices such as replacement of animals and sharing communal areas, vaccination levels, herd size, and farming systems (Nicoletti, 1990; Omer, *et al.*, 2000b; Silva, *et al.*, 2000). Indeed, mixing of herds in communal grazing areas was observed to be associated with increased seroprevalence of brucellosis from 0.7% in 1986 to 3.3% in 1988 in Zimbabwe (Bishop *et al.*, 1994). A higher prevalence of brucellosis infection has also been observed to occur under extensive management systems such as the pastoral system, when compared to other systems (Omer, *et al.*, 2000b; Silva, *et al.*, 2000), and this may be attributed to the effects of communal grazing. Climate, in particular ambient temperature and relative humidity, may also affect the dynamics of the disease. For example, *Brucella* organisms can survive in an aborted foetuses in sheds and in liquid manure for up to eight months, three to four months in faeces, two to three months in wet soil and one to two months in dry soil (Bishop, *et al.*, 1994). Favourable environmental conditions that enhance survival could, therefore, perpetuate transmission of the organisms.

1.3.2.2 Wildlife animals

Transmission of brucellosis among wildlife is highly dependent on species and social behaviours (Hillman, 2002). Transmission rates are greater in highly social animals, especially ungulates. In social ungulates the bacteria are spread through direct contact with discharge from the vagina, aborted foeti, and sexual intercourse. Wild ungulates could also acquire infection by ingesting contaminated pasture (Bishop, *et al.*, 1994) Carnivores such as wolves and foxes are thought to be exposed through the consumption of infected animals, placentae or aborted foeti (Hillman, 2002).

1.3. 2.3 Humans

The occurrence of human brucellosis is assumed to be related to the prevalence in animals and practices that expose humans to infected animals or their products (Jaber, *et al.*, 1999). Poor hygiene coupled with close contact with infected animals and consumption habits are the main contributory factors to the spread of the disease in humans (Jaber, *et al.*, 1999). Humans acquire infection through consumption of contaminated raw milk, milk products, blood and meat (WHO, 1997). Acquiring infection through direct contact is a potential threat to occupational groups such as farmers, veterinarians, butchers, laboratory workers, milkers and inseminators (Dessai *et al.*, 1995; Schussler *et al.*, 1997; Minja, 2002). This could also occur in farmers who assist normal deliveries, attending retained placenta or dystocia cases without using protective clothing. Furthermore, inhalation of the pathogens from dust or accidental self inoculation with *B. abortus* S19 vaccine have also been reported to result in human infections (Ole-Goig and Canela-Soler, 1987; Bishop *et al.*, 1994). Therefore, in the pastoral and agro-pastoral farming systems of northern Tanzania,

livestock keepers are at risk from *Brucella* infection due to close cohabitation under poor hygiene conditions, traditional consumption habits, and handling animals without protective materials.

1.4 Pathogenesis

The establishment of infection is influenced by the size of the infective dose, virulence of the bacteria, and host factors such as innate resistance, age, sex and reproductive status of the animal (Bishop, *et al.*, 1994). *Brucella* organisms gain entry to the body via ingestion, inhalation, penetration through abraded skin, or via the mucous membranes of the pharynx and alimentary tract. The organisms infect both phagocytes and non-phagocytic cells (Corbel, 1999), and the latter localise in the rough endoplasmic reticulum (RER). Although *Brucella* organisms are phagocytised by polymorphonuclear or mononuclear phagocytic cells, they can survive and replicate within these cells without being killed (Corbel, 1999). *Brucella* organisms use several mechanisms to avoid or suppress macrophage bactericidal responses (Corbel, 1999), and these include production of inhibitors such as adenine and guanine monophosphate that inhibit phagolysosome fusion, degranulation and activation of the myelo-peroxidase-halide system, and production of tumour necrosis factor-alpha (TNF-α) (Caron, *et al.*, 1994; Corbel, 1997). The capacity of *Brucella* species to use pathways that avoid TNF-α production during infection may be an attribute of virulence (WHO, 1998). Gamma interferon (γIFN) has also been found to be a potential activator of macrophages, by reducing *Brucella* growth, but it does not alone result in total elimination of the micro-organisms.

Following invasion in the host, the organisms are carried by neutrophils and macrophages to regional lymph nodes where they multiply, resulting in lymphadenitis. Following multiplication of the organisms, bacteraemia follows that may last for several months, and may either resolve or be recurrent (Bishop, *et al.*, 1994). During bacteraemia, *Brucella* organisms are carried intracellularly or free in the plasma and localise in various organs such as the gravid uterus, udder, supramammary lymph nodes, spleen, testes, male accessory sex glands and in synovial structures (Bishop, *et al.*, 1994). The sugar alcohol, erythritol, present in the placenta, has been found to be a strong growth stimulant of *B. abortus,* thus accounting for its localisation in the gravid uterus (Bishop *et al.*, 1994). As the infection assumes a chronic form, bacteraemia becomes intermittent and tends to occur around parturition (Jubb, *et al.*, 1991).

Following *Brucella* infection, both antibody mediated and cellular mediated responses are seen. Following infection, T-cells (CD4 and CD8 subsets) play a key role in cell-mediated protection whereas, smooth lipopolysaccharide (S-LPS) mobilises antibody production (humoral response) (WHO, 1998). The first appearance of antibody is related to the size and virulence of the inoculum and the host susceptibility. The antibody of the IgM class is the first to be detectable in the serum, followed by antibody of the IgG class (Berman, 1981). However, on the average antibody reaches diagnostic titres by four weeks after exposure in heavy pregnant cows and at about ten weeks after exposure in non-pregnant cows (Berman, 1981). Variation on the duration for antibody detection is dependent on the sex, age, stage of pregnancy and the virulence of the organism (Berman, 1981; Radostits, *et al.*, 2000). As the disease advances, the level of IgM wanes and IgG become predominant. It has been shown that humoral reaponse does not provide the main protective immunity, it is the cell-mediated response that plays a major role in the

defensive mechanism against *Brucella* organisms (Bishop, *et al.*, 1994). Claves born from seropositive dams are passively immunised via the colostrum and this interferes with vaccination (Radostits, *et al.*, 2000). Usually the antibodies declines to undetectable levels though few remain immune for a long time (Radostits, *et al.*, 2000).

1.5 Clinical manifestations

1.5.1 Livestock

The incubation period of brucellosis is very variable and has been defined in several ways (a) as the period between exposure and abortion or (b) the period between exposure and the first appearance of clinical disease or (c) the period between exposure and before the first serological evidence of infection can be detected (Bishop, *et al.*, 1994). In cows that eventually abort, the length of the incubation period varies according to the time at which infection occurred. Cows infected at service abort after an average interval of 225 days, whereas those infected at seven months gestation abort around 50 days later (Bishop, *et al.*, 1994). Generally, the incubation period is influenced by size of the infective dose, age, sex, stage of gestation and immunity of the animal (Crawford, *et al.*, 1990; Bishop, *et al.*, 1994).

Clinical findings are dependent upon the immune status of the herd or flock. In highly susceptible groups, abortion storms during the third trimester, retained placenta, metritis and reduced milk production are the major clinical signs though they are not pathognomonic (Ariza, *et al.*, 1992). It has also been reported that about 20% of infected animals do not abort, while 80% of animals that abort as a result of *B. abortus* infection do

so only once (Bishop, *et al.*, 1994). The disease has been associated with infertility in cattle, goats, sheep, dogs and pigs (Corbel, 1988) and abortion in cattle (Swai, 1997; Isloor, *et al.*, 1998; Kubuafor, *et al.*, 2000). Male animals develop orchitis, hygromas and sometimes inflammation of the seminal vesicles.

1.5.2 Humans

In humans, brucellosis has an acute, subacute or chronic course, and the incubation period is usually one to three weeks, however occasionally, it may be several months (WHO, 1997). Irrespective of the course of the disease, the predominant signs are intermittent or irregular fever, backache, headache, anorexia, weight loss, weakness, mental depression and arthralgia (Abram, 1985; Corbel, 1988; Benjamin and Annobil, 1992). Joint pain is common, with the sacroiliac joint being mostly affected during the acute stage. In the chronic stage, the knee joint is most often affected (Lulu, *et al.*, 1988). Localised complications may occur and may involve the cardiovascular, gastrointestinal, genitourinary, hepatobiliary, osteoarticular, spleen, lymphatics, pulmonary and nervous systems (Benjamin and Annobil, 1992; Ghassan *et al.*, 1996; Schussler, *et al.*, 1997; WHO, 1998) resulting in various clinical signs. For example, involvement of the nervous system leads to neuro-brucellosis, a condition characterised by fever, psychosis, headache, behavioural changes, seizures, amenorrhoea and spastic paresis (Yamout *et al.*, 1996).

1.6 Diagnosis

The clinical diagnosis of brucellosis has never been straightforward in either animals or humans (Baily, *et al.*, 1992). Currently, diagnosis is based on clinical observation,

complemented by serology, culture and molecular techniques (Ariza, *et al.*, 1992; Ghassan, *et al.*, 1996; Gallien, *et al.*, 1998). Diagnostic tests for brucellosis have been subdivided into three groups namely, demonstration of *Brucella* organisms, detection of immunoglobulins, and those dependent on allergic reactions (Bishop, *et al.*, 1994; Pouillot *et al.*, 1997).

1.6.1 Tests to demonstrate Brucella organisms

1.6.1.1 Culture

Specimens of choice for culture in animals include foetal membranes, uterine discharges, milk, blood or colostrum from infected animals, and stomach contents, liver and spleen of aborted foeti. The supramammary lymph nodes are the most suitable specimens for isolation of *Brucella,* but retropharyngeal or prescapular lymph nodes may also be collected (Bishop, *et al.*, 1994; Abdel-Hafeez *et al.*, 1995). In humans, blood, cerebrospinal fluid and urine are standard clinical specimens for culture, especially during the acute stage of the disease (Fuerst, 1983; Ghassan, *et al.*, 1996; Corbel, 1999). During the chronic stage, few bacteria will be present to allow successful culture. Complications of this method include the slow growth of *Brucella* organisms (Corbel, 1999), and the considerable risk to health of laboratory personnel (Baily, *et al.*, 1992; Corbel, 1997).

1.6.1.2 Microscopic examination

Modified Ziehl Nelsen staining (Stamp's staining) is used for identification of *Brucella* organisms, but it is worth noting that this colour reaction is not specific to *Brucella*

organisms, as *Coxiella, Chlamydia* and *Norcardia* species also express acid-fast features (Bishop, *et al.*, 1994). Smear impressions can be obtained from those samples destined for culture.

1.6.1.3 Molecular techniques

Molecular biological techniques have the advantage of shortening the time required to identify the pathogens and they may detect organisms directly in clinical specimens. For diagnosis and epidemiological studies of brucellosis, techniques such as the Polymerase Chain Reaction (PCR), Restriction Endonuclease Analysis (REA) and Restriction Endonuclease and Hybridisation have been used (Tenover, 1988; Ghassan *et al.,* 1996), and offer high degrees of sensitivity and specificity (Queipo-Ortuno, *et al.,* 1997; Gallien *et al.*, 1998). However, these techniques are too expensive to be used widely, they are more and appropriate for differential diagnosis rather than for establishing prevalence.

1.6.2 Tests for detection of specific immunoglobulins

There are various serological tests available for measuring antibody following infection. Brucellosis in humans and animals is generally diagnosed by serological methods such as Serum agglutination test (SAT), Complement Fixation test (CFT), Rose Bengal Plate test (RBPT) and Enzyme Linked Immunosorbent Assay (ELISA) (Gallien, *et al.*, 1998; Saravi *et al.*, 1995; Ocholi, *et al* 1996; Minga and Balemba, 1990; Radostits, *et al.*, 2000). The milk ring test (MRT) is used in animals and there are no reports of the method being used in humans (Ahmed and El-Aal, 1996; Mohan *et al.*, 1996).

1.6.2.1 Serum agglutination test (SAT)

Although this technique has been used widely as a routine screening of brucellosis for decades in several countries, it has been shown to have limitations (Mahlau, 1967; Ariza *et al.*, 1992; Madsen and Anderson, 1995; Jiwa *et al* 1996; Swai, 1997). Such limitations include the failure to differentiate natural infections from the effects of vaccination, and failure to detect *Brucella* antibodies following abortion or during early incubation, while the test can also becomes negative during chronic stages of the disease (Corbel, 1988; Bishop, *et al.*, 1994).

1.6.2.2 Complement fixation test (CFT)

Due to its high sensitivity and specificity, this test is regarded as the definitive test for the serological detection of infected animals and humans (Ding, 1993; Bishop, *et al.*, 1994; Batra, *et al.*, 1998; Omer, *et al.*, 2000a). Complement Fixation Test results are rarely complicated by non-specific reactions and unlike the SAT, the titre does not wane as the disease become chronic.

1.6.2.3 Rose Bengal plate test (RBPT)

This test has been used in several countries such as India, Zimbabwe, Ghana and Eritrea for screening livestock, wildlife and human populations (Dessai *et al.*, 1995; Madsen and Anderson, 1995; Kubuafor, *et al.*, 2000; Omer *et al.*, 2001). In these settings false negative results are rare and are usually obtained during early stages of the incubation or immediately after abortion, whereas false positives occur due to the presence of IgM as a

result of S19 vaccination, colostral antibodies in young stock, and cross reaction with other bacteria (Bishop, *et al.*, 1994).

1.6.2.4 Milk ring test (MRT)

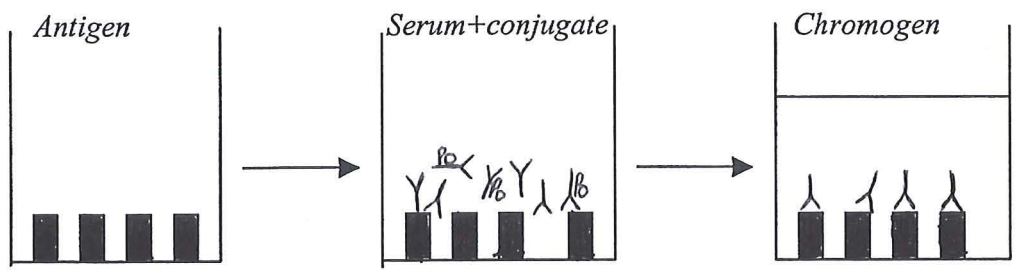
The test is used to detect infected animals on a herd basis or to monitor clean herds (Bishop, *et al.*, 1994). Its sensitivity is low as observed by Vanzini *et al.*, (2001) when compared to ELISA. For example, when sera used for ELISA and milk for MRT were obtained from the same female animals, the former technique revealed more positive animals than the latter one (Ahmed and El-Aal,1996). The test has shown several shortfalls and these relate to low sensitivity, attributed to the presence of mastitis, following vaccination with S19, use of soured milk in the test, and marked changes of ambient temperatures (Bishop, *et al.*, 1994). The test is not applicable in sheep and goats due to the high fat content of their milk.

1.6.2.5 Enzyme Linked-immunosorbent Assay (ELISA)

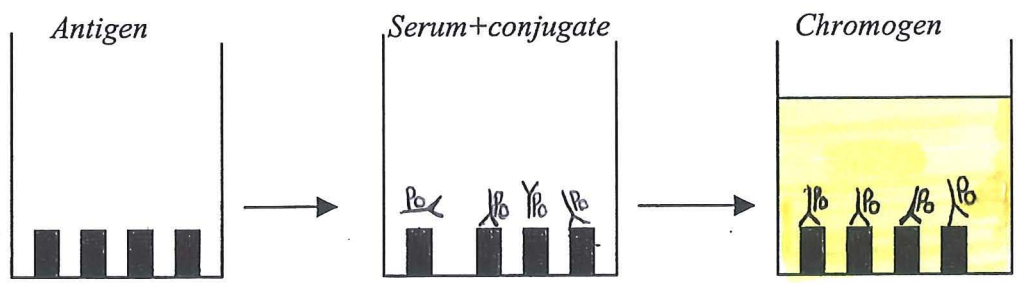
The advent of the ELISA technique has improved the sero-diagnosis of brucellosis (Bishop *et al.*, 1994). The technique was found to be more sensitive than other serological tests (Saravi *et al.*, 1995; Ocholi *et al.*, 1996; Batra *et al.*, 1998). Among the ELISA methods, the Competitive ELISA (c-ELISA) was found to be more robust and easy to perform compared to others (Figure 1.1). The c-ELISA has several diagnostic merits and these include high sensitivity and specificity, ability to differentiate vaccinated animals from naturally infected ones, or those infected with a cross-reacting organisms, and its use in

areas where disease prevalence is low (Nielsen, *et al.*, 1995; Uzal, *et al.*, 1996; Gall, *et al.*, 1998; Sarmatino, *et al.*, 1999; Biancifiori, *et al.*, 2000). Additionally, the c-ELISA can be used on either serum or milk samples from different species (Saravi, *et al.*, 1995; Vanzini, *et al.*, 2001).

Positive sample



Negative sample



Antigen Anti-*Brucella* antibody in the serum Monoclonal conjugate

Note: The amount of substrate hydrolysed is inversely proportional to the antibody present

Figure 1.1: Principles of the Competition ELISA

1.6.3 Brucellin allergic skin test (BAST)

Brucellin allergic skin test is based on a delayed-type hypersensitivity response with a maximum sensitivity at 72 hours post-inoculation. The test is used to complement serological tests for diagnosis of bovine brucellosis, and thus reduce significantly the number of false positive reactions by discriminating brucellosis from other cross-reacting organisms (Saegerman, *et al.*, 1999). The test is superior to RBPT and CFT in terms of its specificity (exceeding 99%), thus it is often recommended for use at the herd level as a confirmatory test in unvaccinated cattle (Pouillot *et al.*, 1997).

1.6.4 Cross-reactions with other micro-organisms

Brucella organisms have antigenic similarities with other bacteria and hence this causes cross-reaction during serology. The common epitope C and 4-amino, 4,6 dideoxymannose in the LPS (Alonso-Urmeneta, *et al.*, 1998; Corbel, 1999) are responsible for the antigenic cross-reactivity with *Escherichia coli* O:157, *Salmonella landau* O:3, *Vibrio cholerae* O:1, and *Yersinia enterocolitica* O:9 (Macmillan, 1990; Corbel, 1999). Several countries have reported such cross-reactions during serological screening (Weynants *et al.*, 1996; Bercovich, 1998), and false positives in addition to false negatives have often limited accurate diagnosis and disease eradication programmes.

1.7 Treatment

1.7.1 Livestock

Treatment of brucellosis in animals is normally not undertaken and treatment trials that have been performed have shown only partial success in eliminating the infection (Radostits, *et al.*, 2000). Some problems have been reported to be associated with the treatment of brucellosis. For instance, the use of antibiotics such as penicillin and oxytetracycline causes L-transformation on the cell wall thereby possibly creating carrier animals, (Bishop, *et al.*, 1994)and affecting future serological detection.

1.7.2 Humans

A number of antimicrobials have been found to be effective against *Brucella*. Since *Brucella* organisms are intracellular organisms, treatment and clearance of the organism usually needs a combination of antimicrobials for several weeks (WHO, 1997). The treatments of choice in acute brucellosis in adult humans involve daily treatment using rifampicin (600-900mg daily) and doxycycline (200mg daily) for a minimum of six weeks (WHO, 1986). Infections with complications such as meningoencephalitis or endocarditis require combination therapy with rifampicin, tetracycline and an aminoglycoside. The worldwide occurrence of multi-drug resistant strains of pathogenic *Mycobacterium* organisms poses an urgent question of alternative treatments for brucellosis, i.e. using other antimicrobials not currently employed for tuberculosis treatment (WHO, 1997). Rifampicin is one of the tuberculosis treatment drugs used under the Direct Observation

Strategy (DOTS) and therefore, its use for brucellosis treatment has to be reviewed especially with the current HIV pandemic.

1.8 Control and eradication

1.8.1 Livestock

Control and eradication programmes based on various strategies have been successful in eliminating brucellosis in several countries (WHO, 1997). Strategies based on the prevention of the spread between animals, monitoring of brucellosis-free herds and zones, elimination of infected animals by test and slaughter, strict control of movement of infected and suspected animals, mass immunization to reduce infection rate, and supporting specific education and training programmes have all received attention in various countries (Abdussalam and Fein, 1976; Bishop, *et al.,* 1994, Ferris *et al.*, 1995; WHO, 1998). Control and eradication of the disease, however, is highly dependent on national strategies, priorities and policies (Bishop, *et al.*, 1994).

Although vaccination has some limitations, especially with live attenuated vaccines, extensive application has been adopted in several countries especially where the disease prevalence was high (Bishop, *et al.,* 1994; Camus, 1995, WHO, 1998). The vaccine preparations currently used in the field are those containing smooth *B. melitensis* Rev.1; rough *B. abortus* strain RB51; smooth *B. suis* strain S2; rough *B. melitensis* strain M111 and smooth *B. abortus* strain S19 (Olsen, *et al.*, 1996; Lord *et al.*, 1998; WHO, 1998). S19, Rev.1 and RB 51 vaccines have been used for the control of the disease in several countries such as South Africa (Bishop, *et al.*, 1994), Israel (WHO, 1999), Cote d'Ivoire

(Camus, 1995) and USA, Mexico and Chile (WHO, 1998) with varying success. Rev.1 vaccine was used extensively in areas where *B. melitensis* infection was high, especially in the Mediterranean and Middle East countries (WHO, 1997; WHO, 1998). The vaccine has shown to induce abortion and it interferes with serological screening when inoculated subcutaneously but if the subconjuctival route is used, interference is reduced significantly (WHO, 1998). The use of RB51 in cattle has been found to be superior than S19, as it does not interfere with conventional serological tests and does not induce abortions (WHO, 1998). S2 and M111 vaccines have been used in China since 1949 in pigs and small ruminants, respectively (WHO, 1998).

In Tanzania, vaccination against cattle brucellosis using S19 was adopted in early 1980's. However, vaccination was confined to government and parastatal dairy farms and no vaccination has been carried out in agro-pastoral and pastoral animals (Kambarage, personal communication, 2003).

1.8.2 Humans

Control and prevention of brucellosis in humans still depends on its eradication or control in animals, practicing good hygienic measures to limit further exposure to infection through occupational activities, and the effective processing of dairy products and other potentially contaminated foods (Corbel, 1999; WHO, 1997). Local customs, habits and beliefs, however, may impede the wide application of such measures in rural areas in many developing countries (Corbel, 1999).

Application of vaccines in the control and eradication of brucellosis in humans has shown unsatisfactory results (Corbel, 1997; Shang, *et al.*, 2002). The use of live vaccines has often provoked unacceptable reactions in individuals. For example, in the USSR and China live attenuated vaccines, *B. abortus* strains 19-BA and 104M were used but tended to be reactogenic and of limited efficacy (Corbel, 1999, Shang, *et al.*, 2002). The recent attempt of developing analogue mutants of another *Brucella species* and the use of *Brucella* nucleic acid in the production of animal and human vaccines offers hope in the control of the disease (WHO, 1998; Corbel, 1999).

1.9 Justification of the study

Brucellosis has a potential impact as a result of (a) livestock production and reproduction losses (b) zoonosis (c) impediments to international trade.

In Tanzania, livestock production plays an important role in household income and food security in the pastoral and agro-pastoral communities that own more than 97% of the national herd. This is despite the traditional way of keeping animals which is characterised by communal grazing, limited pastures especially during the dry period, lack of feed supplementation, and poor or inadequate animal health services, the latter being compounded by the withdrawal of public health services, leaving a vacuum that has facilitated informal delivery of services. Due to these factors, diseases including zoonoses, have continued to be important health constraints in Tanzania.

Brucellosis is one of the diseases which is likely to cause significant socio-economic effects for the traditional livestock sector. It has been suggested that farming characteristics of the sector such as communal grazing resulting in gross contamination of

grazing areas, and the lack of specific disease control strategy such as the use of S19 vaccine, facilitate the establishment of the disease in some localities. The economic implications of the disease include abortion and hence reduced calf crops and replacement stock, and retained placenta accompanied by endometritis that often leads to loss of milk production and added costs of treatment. The public health implication centres on brucellosis in man which, in Tanzania, can be confused with malaria, typhoid fever and other malaria-like syndromes, resulting in possible misdiagnosis and inappropriate treatment. Consumption of raw milk and blood, undercooked meat and handling of aborted materials without protective gear, which is not uncommon in rural settings, suggest that rural communities are at great risk of contracting the disease if the disease is present in domestic animals.

Despite the lack of routine screening of patients with malaria-like syndromes, even when there are anti-malaria drug failures in most hospitals and other health facilities in Tanzania, there are some health facilities in Arusha and Manyara regions which have recognised the presence of brucellosis in communities and have adopted the procedure of routinely screening for brucellosis for most of the persistent malaria-like cases. Evidence of the presence of brucellosis in the same areas (Arusha and Manyara regions) is supported by the results of the seroprevalence studies carried out by Niwael (2001) and Minja (2002) and a demand from communities during tuberculosis studies in the area. Three hospital reports indicated that of 2013 *Brucella* suspect cases examined in 1999, 13% were sero-positive for brucellosis, thus suggesting that the disease may contribute to significant human morbidity in the pastoral and agro-pastoral communities in the two regions (Shirima, 2000 unpublished). As human brucellosis might be expected to follow the pattern of the disease in animals (Jaber, *et al.*, 1999), the presence of the disease in

humans based on existing hospital records indicates that the infection is likely to be present in animals in the two study regions.

Although several studies on brucellosis have been conducted in livestock in Tanzania several important issues still need to be addressed:

(i)The effects of brucellosis on abortion, retained placenta and on milk production have not been quantified.

(ii) The majority of studies conducted in Tanzania did not include small ruminants although they share all resources with cattle and are potentially important sources of *Brucella* infection for people.

(iii) The livestock studies carried out in Tanzania did not identify or quantify risk factors responsible for brucellosis transmission. Although many potential risk factors of transmission for livestock were suggested no specific information on the relative importance of different factors in Tanzania.

(iv) Although brucellosis is a zoonotic disease, none of these studies conducted in Tanzania have linked livestock brucellosis and human brucellosis. Furthermore, there have been no extensive studies conducted in livestock in the northern zone especially Arusha and Manyara regions, despite reports of an increasing occurrence of human brucellosis in the area.

(v) Brucellosis is one of several zoonotic conditions prevalent in Tanzania. Livestock keepers in pastoral and agro-pastoral communities are likely to be more prone to these diseases due to close cohabitation, handling animal cases and their eating habits. The knowledge of the community regarding these diseases, their attitudes and practices that predispose them to zoonoses has not been studied previously in Tanzania, but is important for future public health education and training.

This study was therefore set up to address these outstanding questions and to generate the data to inform policy making and disease control strategies.

1.9.1 Primary objective

To improve the standard of living among pastoral and agro-pastoralists communities in Tanzania through increased public health awareness relating to brucellosis.

1.9.2 Specific objectives

(i) To assess the knowledge, attitudes and practices of rural communities relating to brucellosis and other zoonoses in pastoral, smallholder dairy and agro-pastoral communities.

(ii) To determine *Brucella* seroprevalence in different regions of Tanzania using previously collected sera.

(iii) To determine the seroprevalence of brucellosis in domestic ruminants in Northern Tanzania.

(iv) To determine the seroprevalence of human brucellosis in families keeping livestock.

(v) To identify risk factors associated with transmission of infection in animals in pastoral and agro-pastoral communities.

(vi) To carry out an assessment on the effect of the disease on livestock reproduction and production performance.

(vii) To evaluate the performance of the Rose Bengal Plate Test for field diagnosis in Tanzania.

CHAPTER II

2.0 MATERIALS AND METHODS

2.1 Study areas

The study was conducted in the Arusha region located in the Northern zone of Tanzania between 2001 and 2003 (Figure 2.1) with the study districts shown in Figure 2.2. During the course of the study, the government divided Arusha region into two regions namely, Arusha and Manyara regions in 2002 (Figure 2.1). Following administrative division, Arusha region has five districts namely, Arumeru, Arusha, Karatu, Monduli and Ngorongoro whereas, Manyara region comprises Babati, Mbulu, Simanjiro, Kiteto and Hanang districts. This administrative division did not affect the study districts. The former Arusha region was bounded by Singida and Shinyanga, Kilimanjaro and Tanga, the Kenyan border and Mara and Morogoro in the west, east, north and south, respectively. The region lies between 34.6 to 38.0^0E and 1.8 to 6.0^0S with an altitude ranging from 1000m to 2000m above sea level. The annual average rainfall ranges from 600mm to 1000mm with heavy and long rains occurring between early March and late April and short rains between November and January. The region has potential for agriculture, food and cash crops, livestock, wildlife animals and mining.

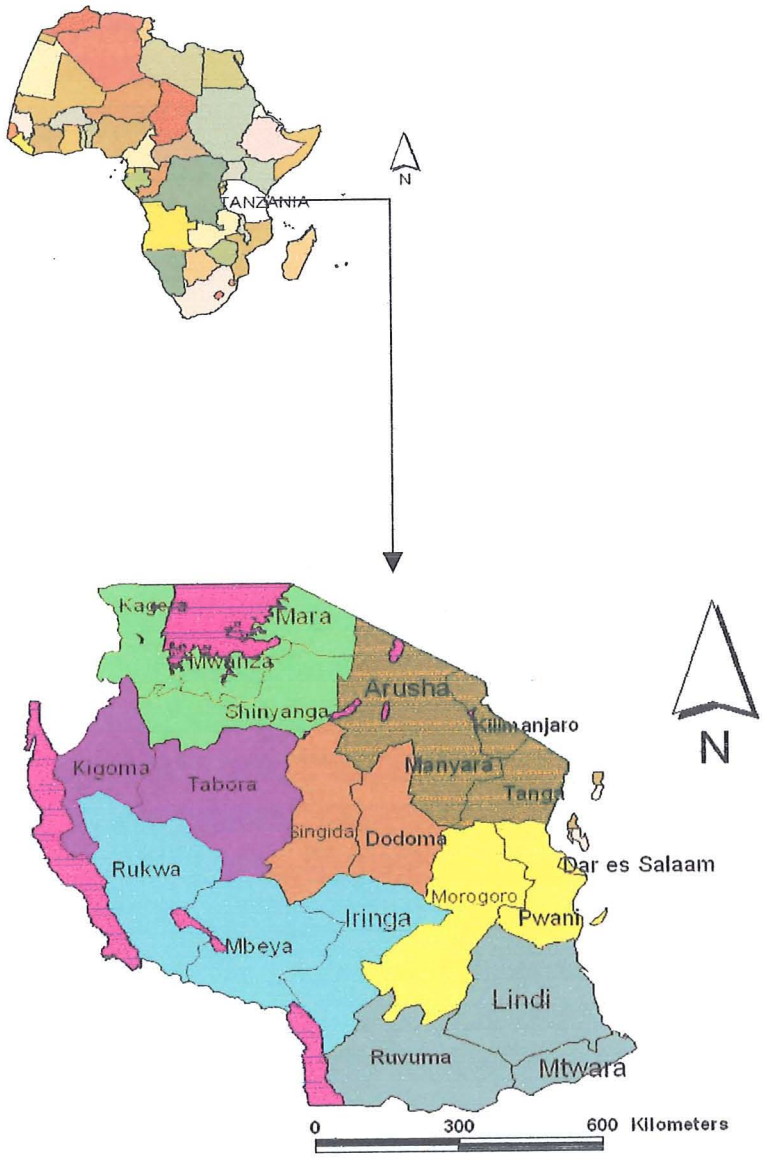
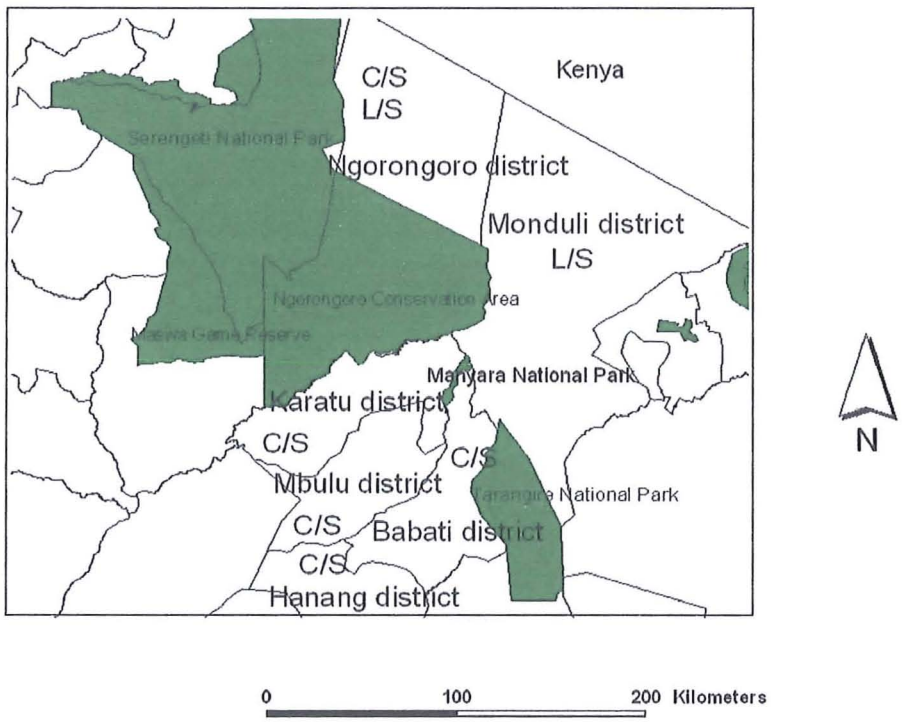


Figure 2.1: Map of Tanzania showing Arusha and Manyara regions in relationship to the surrounding regions



C/S = Cross-sectional study; L/S = Longitudinal study

Figure 2.2 The study districts

In terms of livestock production systems, Arusha region has four major systems namely, pastoral, agro-pastoral, ranches, and smallholder dairy. This study focussed on the former two systems.

2.1.1 Pastoral production systems

The pastoral production system is characterised by owners keeping relatively large herds that graze freely in vast communal lands with watering points. Animal owners keep Tanzania Short Horn Zebu (TSHZ) cattle, sheep and goats and the majority also keep

donkeys for transport and dogs for security reasons. Livestock owners practice a free grazing system, using communal grazing grounds and watering points where cattle and small ruminants graze separately. The majority of herd owners are semi-sedentary with only a few still practicing nomadism. Animals are usually kept in the kraal (boma) at night and young stock of usually less than two months of age share the house with humans. Older cattle are kept in a separate kraal with sheep and goats.

Livestock owners keep relatively large herds and flocks for meat and milk for their families, as a source of savings, and to meet some cultural and social values such as dowry, celebrations, gifts and for social prestige. It is has been estimated that more than 50% of the household income is derived from livestock and livestock products (Thornton, *et al.*, 2002).

2.1.2 Agro-pastoral production systems.

The agro-pastoral production system is characterised by livestock owners keeping relatively small herds and flocks with limited grazing areas and feeding crop residues after harvesting. Animal owners keep TSHZ cattle, sheep and goats and some also keep pigs, dogs, poultry and donkeys for various purposes. The majority of livestock owners are sedentary, practicing free grazing during the rainy season in limited communal areas, and feeding crop residues after harvesting towards the dry season. Herds and flocks are small in number averaging 1-30 animals per household (Thornton, *et al.*, 2002). The majority of herd owners keep animals in the same house with family members with some in the boma or in a separate house.

These animals are kept to supply meat and milk for families, draught power, transport, as a source of savings and to provide cultural and social functions. It is estimated that 10-50% of household income is derived from livestock (Thornton, *et al.*, 2002).

2.2 Zoonoses survey

Two approaches were used to gather information on knowledge, attitude and practices (KAP) relating to zoonoses: (i) Participatory methods involving focus group discussion. (ii) Randomised household surveys of livestock keeping families.

2.2.1 Participatory rural appraisal

The Participatory rural appraisal (PRA) was carried out in two regions of Tanzania namely, Arusha and Iringa. Arusha region is located in the north of Tanzania while Iringa is in the Southern Highlands. The PRA study was conducted between October 2001 and March 2002.

Four districts were involved in the PRA study namely, Ngorongoro, Babati, Karatu in Arusha region and Iringa rural in Iringa region. Fifteen livestock keeping villages were selected and visited. Districts and villages were selected for convenience based on information gained by personal experience of previous researchers and anticipated co-operation from village leaders. Advice was also sought from district livestock officers as to which villages were to be visited.

Three pastoral villages were selected from Ngorongoro district: Enguserosambu, Wasso and Olorien, whereas two agro-pastoral villages, Endallah and Mbuga nyekundu were selected from Karatu district. In Babati district, four agro-pastoral villages were selected: Bermi, Bagara, Gidamar and Managhats. Six smallholder dairy keeping villages: Mkimbizi, Kilolo, Tanangozi, Ihimbo, Lulanzi and Ilula were selected from Iringa rural district.

Prior to the survey, visits were made to each village in order to meet village leaders and to explain the aims of the study and criteria for selecting participants. Village leaders were asked to select two people from each subvillage (kitongoji) to participate in the meeting. In collaboration with village leaders, the date, time and venue for the meeting was arranged at their convenience.

Prior to carrying out the discussion, the district livestock field officer (facilitator) who led the discussions was trained in the way to best guide the group. Where necessary the group was prompted to initiate responses. Focus group discussions (FGD) were conducted in all villages using the national language (Kiswahili) or the local tribal language, depending on the composition of the group. If the vernacular was used, the facilitator translated this to allow recording by team members. This technique was used to gather information on various zoonoses, the clinical signs of each disease in animals and humans, routes of transmission from animals to humans, family activities and consumption of animal products. Responses were recorded by team members without interfering with the discussion and team members only intervened when clarification was required.

2.2.2 Human cross-sectional questionnaire survey

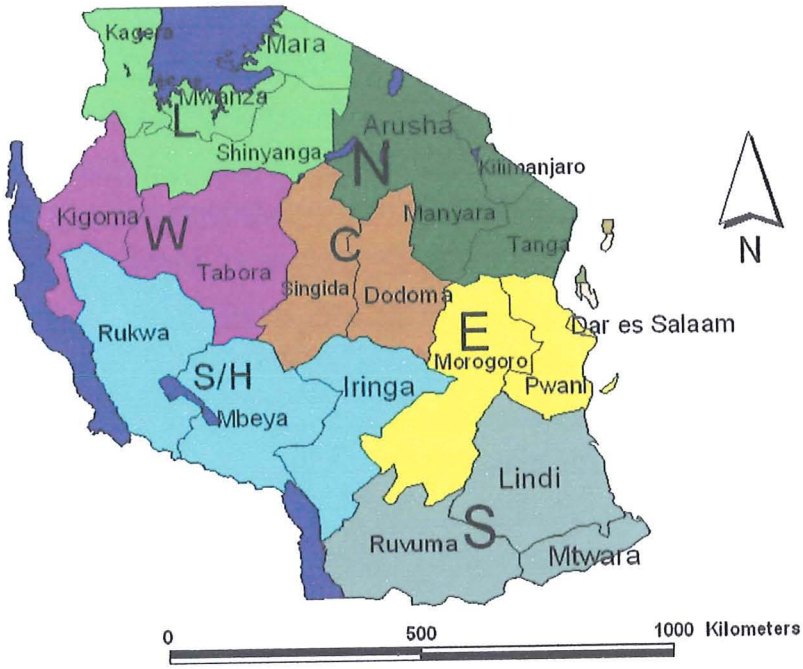
Multistage random sampling was used to select households keeping livestock whereas respondent was obtained by convenience. The human questionnaire covered awareness on various zoonoses, knowledge on transmission of diseases, clinical signs, livestock associated with zoonoses, livestock related activities and food consumption habits. The zoonoses section was developed based on the most frequently identified zoonoses during the PRA. The human questionnaire was conducted personally with 101 households. This interview took about 20-30 minutes. Public health awareness leaflets were also prepared based on the PRA information to raise awareness on zoonoses and these provided after the questionnaires had been delivered. The questionnaire and the leaflet are included in the Appendix 1 and 2.

2.3 Preliminary brucellosis serosurvey in Tanzania

Serum samples were obtained from a serum bank kept at the Sokoine University of Agriculture (SUA), Morogoro and Tanzania National Parks (TANAPA) laboratory, Serengeti, Tanzania. Samples were collected from livestock and wildlife animals between 2000 and 2001. A total of 3048 cattle, 93 small ruminants and 90 wildlife sera were made available for analysis. The livestock sera were collected from different farming systems in various agro-ecological zones of Tanzania (Table 2.1), through various projects conducted in each zone. In the Southern Highland zone, samples were collected by the Department for International Development (DFID)-funded Mastitis Project, whereas in the eastern zone, sera were collected by the Tanzania Agricultural Research Project (TARP 11). In the northern zone, sera were collected with assistance from the DFID-funded Bovine

Tuberculosis Project. Livestock sera from the lake zone were collected with assistance from the Project Life Lion, whereas sera from central zone were made available by the Veterinary Investigation Centre (VIC) based at Mpwapwa (Figure 2.3). With the exception of samples from central zone where purposive sampling was used, samples from other zones were collected at random. Samples from the northern and lake zones were collected using multistage sampling of herds and individual animals were selected from the herd by convenience. From the Eastern and Southern Highlands zones, animals were selected using multistage cluster sampling with the herd selection criteria being based on a maximum 10 dairy animals owned.

The wildlife sera were collected opportunistically from the Serengeti-Ngorongoro ecosystem probably as part of rinderpest surveillance and made available with the permission of Chief Veterinary Officer, Tanzania National Parks (TANAPA).



C = Central zone, E = Eastern zone, L = Lake zone, N = Northern zone, S = Southern zone, S/H = Southern Highlands, W = Western zone

Figure2.3. Agro-ecological zones in Tanzania

Table 2.1: The distribution of available cattle sera based on agro-ecological zones and farming systems of Tanzania in 2000/2001

Zones	*Projects involved in collecting serum*	*Farming systems*				
		Smallholder	*Agro-pastoral*	*Pastoral*	*Ranch*	*Total*
Southern highland	DFID-funded Mastitis Project	58	0	0	0	58
Eastern	Tanzania Agricultural Project (TARP II)	874	0	0	0	874
Northern	DFID-funded Project Bovine Tuberculosis Project	664	337	165+ 93*	0	1259
Central	Veterinary Investigation Centre(VIC)-Mpwapwa	0	0	0	403	403
Lake	Project Life Lion	0	547	0	0	547
Total		1596	884	258	403	3141

+Cattle screened from pastoral herds

**Small ruminants screened from pastoral flocks*

2.4 Cross-sectional survey

2.4.1 Study villages and animal sampling

The cross-sectional study was carried out from May 2002 to July 2003. Livestock-keeping households were selected by a process of multi-stage random sampling. The sampling frame comprised a list of all villages in the study area (n=285), which was made available at district livestock offices. A random sample of 32 villages was selected using a table of random numbers. Among these, 20 were agro-pastoral and 12 pastoral villages. In

each village multistage sampling was used to select at random two sub-village administrative units, (known as *kitongoji*). A ten-cell leader (*balozi*), a leader of ten or more households, was selected at random from each sub-village and all livestock-keeping households were identified. Finally, two livestock-keeping households were randomly selected from each ten-cell unit. The initial sampling procedure (involving 12 villages) involved selection of two households from one balozi within each village. This achieved a wide geographic coverage but was considered to be too time-consuming and the sampling procedure was therefore revised to include two households from each of two ten cell units. The livestock sample size was estimated to provide 80% power with 95% confidence. Based on the previous reported mean prevalence of brucellosis of 5%, the sample size was calculated as described by Martin *et al.*, (1987) to obtain the total number of animals to be screened from each selected household.

2.4.2 Livestock and human sampling

2.4.2.1 Livestock data and sample collection

Following household identification an initial visit was made to arrange the forthcoming activities with the household owner. On the day of the visit, all animals in the household were collected and random number allocation was used to choose animals for screening. Despite preliminary attempts to sample animals at random within the herd, this was difficult to achieve without a systematic method of restraint, such as a crush. For most of the herds, blood samples were therefore collected from animals restrained by the householders without systematic or truly random selection of individuals. Each animal was bled from the jugular vein using a sterile needle and a plain vacutainer (Becton and

Dickinson, UK). The animal was ear tagged using a metal tag (Ketchum, UK) for subsequent identification. The blood sample was labelled using the tag number assigned to each individual animal. Individual animal information was obtained from the herd owner by personal questionnaire at the time of sampling. The information collected included history of abortion, retained placenta, the past two calving dates and current reproductive status. The dentition and sex of each animal was recorded. Age class was categorised based on the number of permanent incisors present as 0 = no permanent incisor pairs (approximately <1.5 years), 1 = one permanent incisor pairs (approximately 1.5-2 years), 2 = two permanent incisor pairs (approximately 2.5-3 years), 3 = three permanent incisor pairs (approximately 3.5-4 years), 4 = four permanent incisor pairs (>4 years). Animals with permanent incisor pairs from 0-2 were classified as young animals whereas 3-4 pairs were classified as adult (mature) animals. The individual animal information questionnaire used is presented in Appendix 3.

Milk samples were collected from a proportion of lactating animals, with collection from each of the four teats pooled in one sterile container. It was difficult to obtain milk samples from all lactating animals as intended because women often milked cows early in the morning or calves had been allowed to suckle the milk before arrival of the research team. The milk samples were kept in a cool box immediately before transportation for storage at an approximately -20°C.

2.4.2.2 Human data and sample collection

Bleeding of human subjects was carried out with ethical clearance from the Ministry of Health, Tanzania. In each livestock-keeping household, family members were approached

to identify volunteers for blood sampling following discussions about the purpose of the project and the potential of the brucellosis problem in the region. Prior to bleeding the medial site of the elbow region was disinfected using cotton wool soaked in methylated spirit (Bell Chemicals Co. Ltd. Dar es Salaam). Blood was aseptically collected from the brachial vein using a disposable 5ml syringe (Young Wood Co-operation, Korea) by a medical personnel. The blood was immediately transferred into a plain vacutainer and assigned an identification number, and kept in a tray for serum separation.

2.4.3 Risk factors associated with brucellosis in livestock

A questionnaire survey was developed through discussions with various people including researchers with experience in conducting research to detect possible ambiguities and defects. The questionnaire was pre-tested in pastoral and smallholder dairy households in Monduli district, Arusha region, before the final version was developed. The livestock questionnaire covered a wide range of topics including herd management practices, knowledge and awareness of livestock brucellosis, livestock movement and interactions with wildlife animals (Appendix 4).

The livestock questionnaire survey was conducted by personal administration in 104 households. The interviews were conducted following bleeding of livestock and humans. The interview was conducted with one family member who was knowledgeable about the herd and/or flock. The information collected included retrospective information over a period of one year. Each interview took about 30-40 minutes. The geographic location of each household was recorded using a hand-held Garmin® Global Positioning Systems (GPS).

2.5 Longitudinal study

2.5.1 Selection of households

The study was conducted in pastoral households and one beef ranch of Arusha region for a period of 12 months, from September 2002 to December 2003. The study was conducted to monitor infection dynamics and to assess the impact of infection in livestock. The criterion used to select herds for the longitudinal follow-up was any household with a FRBPT seroprevalence of ≥10% during the cross-sectional screening (Chapter V). Four households were selected from the cross-sectional study on the basis of these criteria. These were pastoral herds located at Soitsambu, Oloipiri, Malambo and Esere villages in Ngorongoro district. These herds had a total of 299 animals for longitudinal follow up. Another two herds with a total of 190 animals were selected from Manyara beef ranch and Alkaria village in Monduli district. The latter herds did not originate from the cross-sectional study but were enrolled due to a high sero-prevalence (≥10%) of brucellosis during the year of this study.

2.5.2 Data collection

Animals were bled at every three months for a duration of twelve months. At each visit, information at the individual animal and the herd level was obtained from the owner. Individual animal information included cases of abortion, retained placenta following normal delivery or abortion, the cost of treating retained placenta and pregnancy status. Replacement of animals which dropped out of the study was carried out by bleeding selected replacements from the herd and/or flock. Newly recruited animals were tagged for

identification. Individual animal information was based on owners' recall over the previous 3-month period. However, not all animals lost to follow up were replaced for several reasons. A livestock field officer was assigned to visit the herd every month to collect individual animal information which was compiled every three months.

2.5.3 Calf growth rate

Seventy nine calves were enrolled in the study. The heart girth was measured in centimeters using a tailor's measuring tape. Calves stood on all four legs while restrained. The measuring tape was placed around the animal just behind the hump and forelegs, and heart girth measurements were taken. Each calf was bled to establish their brucellosis serostatus and matched with the respective dam serostatus. Heart girth measurements were carried out at three monthly intervals.

2.5.4 Collection of placenta and aborted material.

Initially it was anticipated that placental materials would be collected from all herds in the longitudinal study. It was possible to do this in only two herds due to limited cold chains in most areas. Sterile bottle containers were provided for storage of placental materials. Protective materials including gloves were provided for safety reasons during collection of aborted and placenta materials. One herd was provided with a liquid nitrogen container for keeping these samples until collection. Samples from the second herd were collected and taken immediately to the district livestock office where the samples were kept at an approximately -20^{0}C. These samples were packed at SUA according to VLA guidelines and sent to VLA, Weybridge for culture.

2.6 Blood processing

Blood samples collected during the cross-sectional and longitudinal studies were processed on the same day of collection. Blood samples were left at ambient temperature for at least 30 minutes after collection to avoid problems of albumin coagulation that prevents serum formation during centrifugation, especially with small ruminant blood samples. In the field, these samples were centrifuged at 3022g for 5 minutes using a Mobile spin centrifuge (Vulcan Technologies, USA). Tubes were removed and serum decanted into eppendorf tubes (Eppendorf-Netheler-Hinz GmbH, Hamburg Germany) in duplicate. All livestock and human sera were kept in the cool box after FRBPT and transported for storage at an approximately -20^{0}C.

2.7 *Brucella* serology

2.7.1 Rose Bengal Plate Test

The RBPT is a rapid agglutination test that is used as a screening test for the detection of antibodies to brucellosis in livestock, wildlife and humans. The antigen used in the study was *Brucella abortus* Rose Bengal-stained antigen kindly donated by Veterinary Laboratory Agency (VLA) Weybridge, UK (batch numbers 269 and SG276). The antigen was used in the field as Field Rose Bengal Plate Test (FRBPT) and in the laboratory as Laboratory Rose Bengal Plate Test (LRBPT).

Briefly, a 40-well Rose Bengal plate was used for the test. Using a disposable glass Pasteur pipette one drop (approximately 30 μl) of serum was placed on each well of the plate.

After warming the Rose Bengal antigen at room temperature for 30 minutes, one drop was drawn using a disposable glass Pasteur pipette and placed alongside the serum on the plate. The serum and antigen were mixed thoroughly using an applicator stick and the plate rocked gently to allow mixing. After four minutes, the plate was examined for agglutination in good light (Figure 2.4). Any degree of agglutination was taken as positive and absence of agglutinates was considered to be negative. The results were recorded and the plate washed with water and methylated spirit and allowed to dry before being re-used.

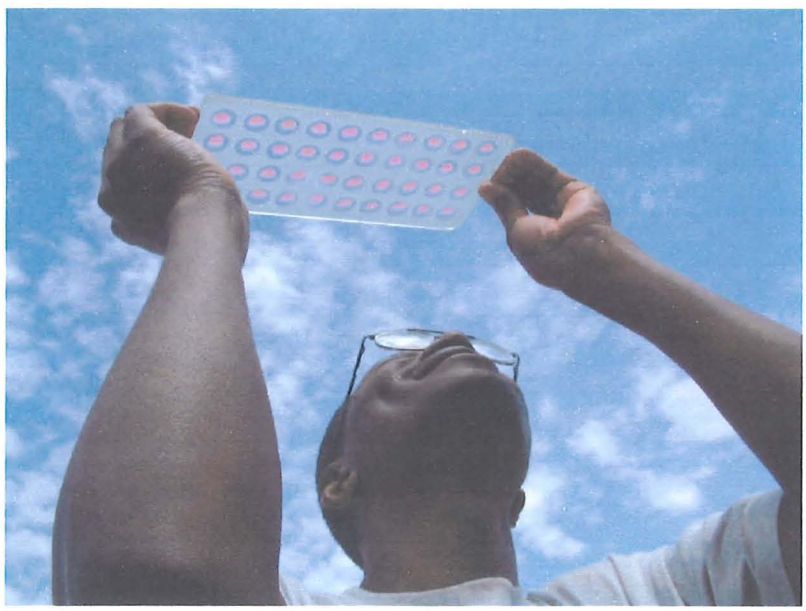


Figure 2.4. Rose Bengal Plate test examination in the field by the author

2.7.1.1 Preliminary brucellosis serosurvey

The RBPT was carried out at SUA, Morogoro and TANAPA laboratory based at Serengeti National Park. A total of 3141 livestock and 24 wildlife sera were subjected to RBPT at

SUA and 66 wildlife sera were tested at TANAPA laboratory in Serengeti based on the procedure described above in section 2.7.1.

2.7.1.2 Cross-sectional serum samples

The FRBPT was performed as described in 2.7.1 on all livestock sera on the day of sampling and feedback was given to respective herd owners on the same day.

During the cross-sectional study, human sera were also collected and analysed in the field using the FRBPT. Symptoms if present were recorded from seropositive individuals. Those with clinical signs and symptoms consistent with brucellosis were given a written note to seek medical attention.

2.7.1.3 Longitudinal serum samples

Livestock sera were analysed in the field, during the first visit using RBPT antigen. Feedback was given to household owners on the same day.

2.7.2 The Competitive Enzyme Linked Immunosorbent Assay (c-ELISA)

With the exception of sera for the preliminary serosurvey, all sera from the cross-sectional and longitudinal surveys were inactivated in a 56^0C water bath for 30 minutes, packed and sent to the VLA for c-ELISA analysis which is considered as the gold standard test for diagnosis of brucellosis.

(a) Testing procedures

The procedure employed for c-ELISA testing for brucellosis was according to VLA protocol (Perret, *et al.*, 2001). Briefly, a 96-well polystyrene microtitre plate pre-coated with *B. melitensis* LPS antigen was used. Using a single channel micropipette 20μl of each test serum was added to polystyrene microtitre wells in duplicate except wells in column 11 and 12. Twenty microlitres of the positive control antisera from VLA was dispensed into the first six wells of column 11 and 12 and 20μl of the negative control antisera from VLA was dispensed into the last six wells. One hundred microlitres of the conjugate buffer was added to all wells. The plate was covered with a lid and incubated at room temperature for 30 minutes on a rotatory shaker at 160 revolutions per minute (rpm). Thereafter, plates were rinsed five times and thoroughly dried by tapping firmly onto an absorbent towel. One hundred microlitres of substrate-chromogen solution was dispensed onto each well, covered and left to react for 15 minutes at room temperature. After the reaction, 100μl of the stopping solution was dispensed to each well and the plate read within 10 minutes.

(b) Interpretation of results:

Before the plate was measured by ELISA reader, visual observation for any colour development was undertaken. Lack of colour development indicated that the sample tested was positive while the test was considered negative if an orange colour developed.

By using the ELISA reader Multiscan RC Version 6.0 (Labsystems, Helsink Finland) at 450nm, the plate results were considered invalid if any of the following applied:

(i)The binding ratio was less than 10.

For c-ELISA binding ratio (BR) is given by:

$$\frac{\text{Mean of the 6 negative control wells}}{\text{Mean of the 6 positive control wells}}$$

(ii) The optic density (OD) of the mean of the 6 negative ODs was less than 0.70. The optimal mean negative OD is 1.0.

(iii) The OD of the mean of the 6 positive wells was greater than 0.10.

(iv) The mean OD of the four conjugate control wells was less than 0.70.

The cut-off value for c-ELISA positivity was based on the conjugate control where the cut-off was taken as 60% of the mean of the OD of the 4 conjugate control wells. Any test sample giving an OD equal to, or below this value, was considered positive. All results were expressed as a percentage of the conjugate control and referred to as percentage positive values (pp values).

2.8 Cultivation of *Brucella* organisms and diagnostic evaluation

2.8.1 Cultivation of Brucella organisms

A total of 375 samples (180 blood, 169 milk and 26 placenta samples) were collected for culture during the cross-sectional and longitudinal studies. All samples were sent to VLA, Weybridge for culture isolation of *Brucella* organisms. Farrell's modified serum dextrose agar was used as a selective medium for *Brucella* organism growth. Antibiotics and antimicrobial agents were added to the media to suppress fast growing organisms.

Serum Dextrose Agar (SDA) was used for sub-culturing. Culturing, identification and typing were carried out according to the protocol of Corbel *et al* (1983).

2.8.2 Diagnostic test evaluation

During the cross-sectional study, a total of 3387 serum samples from domestic ruminants were collected and tested using the RBPT in the field while 3288 samples were tested in the laboratory using laboratory Rose Bengal Plate Test (LRBPT) and all samples were tested using c-ELISA at VLA. Of the samples tested in the field, 1948 serum samples were collected from pastoral animals and 1439 were collected from agro-pastoral animals.

2.9 Data storage and analysis

Data were entered in a Microsoft Excel 97 (1993) spread sheet. Some of the variables collected from interviews were summarised using narrative text, whereas questionnaire findings were coded and analysed using descriptive statistics. The Chi-square test was used to compare two or more proportions to determine associations and statistical differences. The Fishers exact test was adopted when one or more of the expected cell values were less than 5. The strength of the association between risk factors and brucellosis status was examined by odds ratio (OR) and 95% confidence intervals (95% CI) values.

OR was estimated as the ratio of the odds of disease in exposed individuals to the odds in those unexposed (Thrusfield, 1995). The attributable risk was calculated as the difference between the incidence of disease in exposed animals and the incidence in unexposed animals **[a/(a+b)]-[c/(c+d)]**. The incidence of the disease was calculated as described by Thrusfield, (1995) and Woodward (2005).

The incidence = Number of new cases in the three months period

(Number of domestic ruminants at risk at start of the period + Number of domestic ruminants at risk at the end of that period)/2

The longitudinal life table was developed to estimate the probability of an event at a given period (q_t) , to estimate the probability of surviving at a given period (p_t) and to estimate the probability of surviving from baseline to the end of the study period (s_t) (Woodward, 2005).

The q_t values are risks given by e_t/n_t where **et** = number of c-ELISA seropositive animals at three months interval and n_t = number of c-ELISA seronegative animals at the end of three months. The p_t is given by $1- q_t$ whereas s_t is given by $p_0p_1p_2....p_t$.

The heart girth measurements between calves suckled from *Brucella* positive and negative dams were compared using Wilcoxon test.

The diagnostic sensitivity and specificity and, predictive values for RBPT were calculated as described by Thrusfield (1995), whereas the overall measure of sensitivity and specificity of a test was determined by calculating the Youden's index. The agreement between FRBPT and LRBPT was determined using Cohen's kappa (Woodward, 2005). The Youden's index (Y) is given by **Se+Sp-1**. Cohen's kappa is given by **(Σa-ΣEf)/N-ΣEf)** where Σa is the total number of agreements by summing the values in the diagonal cells (true positives and true negatives), ΣEf is the sum of the expected frequencies for the number of agreement that would have been expected by chance and applied only to the diagonal cells, and N is the total number of observations. The true prevalence (TP) was

estimated by using the sensitivity and specificity of ELISA test given by, $\mathbf{TP = (P^T + Sp) - 1/(Se+Sp)-1}$, where P^T = test prevalence.

The association between c-ELISA results and animal and household level explanatory variables was analysed using a univariable binomial regression model. Generalised linear mixed effects models for binary outcome of the cross-sectional data at the individual level were fitted using EGRET for Windows software (Gogte, *et al.*, 1999). To analyse household effects, animal and farm level datasets were merged before copying them into the EGRET programme. Logistic regression with a random effect model was then fitted to assess household effects. Data analysis was performed by fitting a logistic binomial regression for distinguishable data using the modified Newton Raphson algorithm procedure.

A final multivariate model was fitted using a forward stepwise procedure. Variables with likelihood statistic ratio of less than 0.2 were selected for multivariate analysis.

Maps were drawn employing Arc-view software (1992).

CHAPTER III

3.0 ZOONOSES SURVEY IN LIVESTOCK KEEPING COMMUNITIES IN TANZANIA

Abstract

A Participatory Rural Appraisal (PRA) and a cross-sectional study were carried out to assess community knowledge, awareness and practices relating to zoonoses in various livestock production systems in Tanzania. The PRA was conducted in pastoral and agro-pastoral communities in Arusha region and smallholder dairy households in Iringa region using focus group discussion techniques. The cross-sectional study was conducted in pastoral and agro-pastoral communities in Arusha region using randomised household questionnaires focusing on the major four zoonoses identified from the PRA study.

Nineteen diseases were reported as zonooses by respondents during the PRA with rabies, tuberculosis, anthrax and brucellosis as the most frequently diseases identified in all farming systems. Rabies was identified in 100% of village responses and anthrax, tuberculosis and brucellosis identified in more than 80% village responses. Other conditions identified by respondents as zoonoses were foot and mouth disease (FMD), *C. bovis*, *C. cellulosae*, tetanus, mange, plague, orf and typhus fever.

Except for rabies, clinical signs of zoonoses in animals and humans were variably reported among farming systems. Respondents were more knowledgeable about human clinical

signs than animal clinical signs. No respondents were able to describe clinical signs of brucellosis in animals.

Ingestion was recognised as a major route of transmission of zonooses in all systems but consumption of raw meat, milk and blood varied between farming systems. Most livestock related activities were performed by women except in smallholder dairy where all family members or hired animal carers were responsible for these activities.

Findings from the cross-sectional study were broadly similar with respect to recognition of zoonoses, clinical signs and animals responsible of transmitting the infection to humans. In pastoral and agro-pastoral households, 22% of households consumed unboiled soured milk while blood was consumed in 71% of the households. Manure handling and milking were performed mainly by women whereas slaughtering was mainly performed by men.

Despite some knowledge of zoonoses and awareness of routes of transmission, household activities are likely to expose them to an increased risk of contracting zonooses.

3.1 Introduction

Animal health and human health are inextricably linked. People depend on animals for nutrition, socio-economic development and companionship, yet animals can transmit many different diseases to humans. Diseases transmitted from animals to humans are termed zoonoses and some of them are potentially devastating. According to the WHO, (1959), zoonoses are defined as diseases and infections which are naturally transmitted between vertebrate animals and man. However, Palmer *et al.*, (1998) challenged the WHO definition by saying that not all conditions are naturally transmitted. Such conditions include unnatural opportunistic infections of severely immunocompromised patients, xenotransplantation and intoxications.

For people who are highly dependent on livestock, livestock diseases, water, feed supply and herd security usually feature as important concerns. Livestock diseases such as tuberculosis, anthrax, rabies and brucellosis cause significant losses in terms of livestock production and reproduction (Radostits, *et al.,* 2000) and pose a threat to public health. In Tanzania several factors that differ between different communities, may facilitate transmission of zoonoses. Such factors includes close contact between animals and humans, intensification of animal production in urban and peri-urban areas, inappropriate eating habits and poor policies related to disease control programmes. Following the rapid expansion of the smallholder dairy sector in urban and peri-urban areas of Tanzania (Swai, 1997), both rural and urban communities are at high risk of contracting zoonoses through attending animals and consumption of animal products. Furthermore, Kambarage (2004) suggested that poverty and poor knowledge about zoonoses among livestock keepers and

consumers are the underlying problems for continued and re-emergence of zoonoses. For example, an increased prevalence of porcine cysticercosis observed in Mbulu district has been attributed to lack of latrines and this was linked to poverty and poor knowledge on the life cycle of the tapeworm (Ngowi, *et al.,* 2001; Kambarage, 2004). Government efforts to control major zoonoses such as rabies, anthrax and brucellosis have not been practiced in Tanzania. Livestock diseases are controlled only to a limited extent as veterinary services are privatised and drugs or vaccines are neither readily available nor easily affordable for the poor rural livestock keepers. Under such circumstances zoonotic diseases that are easily transmissible from livestock to humans may play an important role as a contributing factor to poor human health and poverty.

During the initial phase of the project, a scoping study was conducted to gather information on zoonotic diseases from animal keeping communities in Tanzania using Participatory rural appraisal (PRA) methodology which was later compared by a cross- sectional semi-structured questionnaire survey. The rationale was to collect data on community knowledge, awareness and local perceptions on zonooses from various livestock production systems using two different methodologies.

3.2 Materials and methods

Both the PRA and cross-sectional studies were conducted in livestock keeping communities. The PRA was conducted in pastoral, agro-pastoral and smallholder diary households while the cross-sectional study was conducted in pastoral and agro-pastoral households only. Focus group discussions were used to collect data during the PRA,

whereas semi-structured questionnaires were used during the cross-sectional survey. The sampling technique, arrangement and discussion procedures are detailed in Chapter II, section 2.2 of the general methodology. During the discussion, respondents were prompted where necessary, especially at the beginning of specific topic to initiate the discussion.

Data was handled using Microsoft Excel (1993) spread sheet 97. Proportions, percentages and bar charts were produced using Microsoft Excel. Univariable analysis for cross-sectional data between knowledge in identifying zoonoses, animals associated with zoonosis, clinical signs in animals and humans consistent with the definition of Acha and Szyfres, (2001) as an outcome variable and farming systems were carried out using logistic binomial regression (R Software, version 1.9.1; 2004).

3.3 Results

3.3.1 Knowledge, attitudes and practices

3.3.1.1 Participatory rural appraisal at village level

3.3.1.1.1 Zoonoses

Nineteen zoonotic conditions were identified by respondents, with rabies, tuberculosis, anthrax and brucellosis most frequently identified in pastoral, agro-pastoral and smallholder dairy farming systems by overall village response (Table 3.1). In the case of rabies, 100% of village responses identified this as a zoonotic disease, while more than 80% of village

responses identified brucellosis, tuberculosis and anthrax as zoonoses occurring in these communities.

Foot and Mouth Disease (FMD) was identified as a zoonosis in pastoralist communities where respondents from all three villages linked clinical signs of disease in humans with outbreaks of FMD in livestock. Plague was identified as a zoonosis in one village in the agro-pastoral and in two villages in smallholder farming systems. Mange was identified in two villages, one each from pastoral and smallholder farming systems. Parasitic diseases were also identified by respondents as zonooses. *Cysticercus bovis* (*C. bovis*), the cystic stage of the tapeworm *Taenia saginata* (*T. saginata*) in humans, was identified by 60% village responses with respondents in 5 of the 6 smallholder villages mentioning this condition. *Cysticercus cellulosae* (*C. cellulosae*), the cystic stage of the tapeworm *Taenia solium* (*T. solium*) in humans, was identified by only 13% of village responses in the smallholder farming system (Table 3.1).

Table 3.1: Diseases identified as zoonoses by respondents through focus group discussions

Conditions considered as zoonoses	***Pastoral villages (n=3)***	***Agro-pastoral villages (n=6)***	***Smallholder dairy villages (n=6)***	***Overall village response (%; n=15)***
Rabies	3	6	6	100
Tuberculosis	2	6	6	93
Anthrax	3	4	6	87

Brucellosis	3	6	3	80
Cysticercus bovis	1	3	5	60
Plague	0	1	2	20
FMD	3	0	0	20
Mange	1	0	1	13
Cysticercus cellulosae	0	0	2	13
Tetanus	0	1	0	7
Typhus fever	0	1	0	7
Orf	0	0	1	7
Cancer	0	0	1	7
Mastitis	0	0	1	7
Malaria	1	0	0	7
Allergies	0	0	1	7
ECF	0	0	1	7
Trachoma	0	0	1	7
Typhoid fever	0	1	0	7

Of the 19 conditions identified, the last seven conditions listed in Table 3.1 are not zoonotic according to WHO definitions (WHO, 1959), including cancer, mastitis, malaria, allergies, East Coast Fever (ECF), trachoma and typhoid fever.

Respondents identified five domestic species as being involved in transmission of zoonosis to humans, including cattle, goats, sheep, dogs and pigs (Figure 3.1). Cattle were identified

as being associated with five zoonoses including tuberculosis, anthrax, brucellosis, *C. bovis* and FMD. Four of the five diseases identified as zoonoses in cattle, were also identified by respondents as being associated with goats, however, no respondents identified brucellosis as a zoonosis linked with goats and sheep. In sheep, only tuberculosis and anthrax were identified as zoonoses. Over 90% of village respondents identified rabies as a zoonosis associated with dogs. The only zoonosis associated with pigs was *C. cellulosae.*

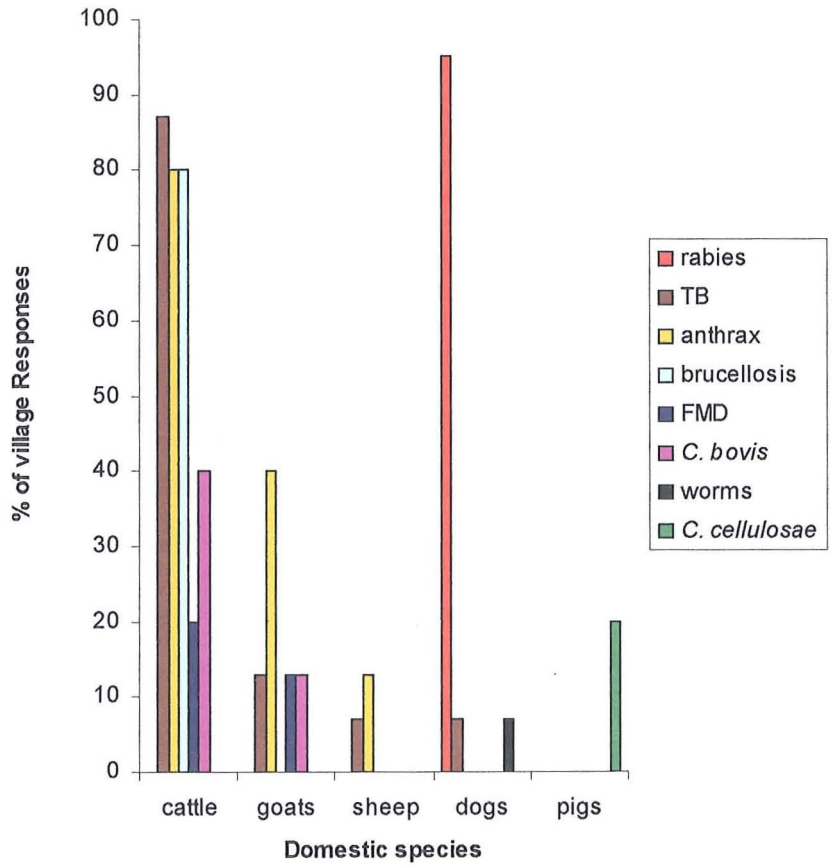


Figure 3.1: Domestic animals associated with zoonoses as identified by respondents during the PRA survey

3.3.1.1.2 Clinical signs of zoonotic diseases in animals and humans

Madness was identified as a clinical sign of rabies in dogs by 100% of village responses, while salivation was only identified in one agro-pastoral village (Table 3.2a).

The village response from pastoral and smallholder dairy farming systems did not identify any clinical signs associated with tuberculosis in animals, whereas two villages in the agro-pastoral farming system identified coughing and emaciation as clinical signs of tuberculosis. No pastoral respondents identified clinical signs associated with anthrax in animals, whereas blood oozing was a pathological feature identified in one smallholder village. Sudden death, swollen abdomen and lack of rigor mortis were clinical signs reported to be associated with anthrax by two village groups in the agro-pastoral sector. No respondents were aware of clinical signs associated with brucellosis in animals (Table 3.2a).

Table 3.2a: Clinical signs and pathological changes associated with zoonoses in animals as identified by respondents during the PRA survey

Disease/signs	***Pastoral villages***	***Agro-pastoral villages***	***Smallholder dairy villages***	*Overall village responses (%)*
Rabies	(n=3)	(n=6)	(n=6)	(N=15)
Madness	3	6	6	**100**
Salivation	0	1	0	**7**
Tuberculosis	(n=2)	(n=6)	(n=6)	(N=14)
Coughing	0	2	0	**14**

Emaciation	0	2	0	**14**
Anthrax	(n=3)	(n=4)	(n=6)	(N=13)
Sudden death	0	1	0	**8**
Swollen abdomen	0	1	0	**8**
No rigor mortis	0	1	0	**8**
Blood oozing	0	0	1	**8**
Brucellosis	(n=3)	(n=6)	(n=3)	(N=12)
None	0	0	0	**0**

Madness was identified by 100% village responses as a clinical sign associated with rabies in humans, whereas death was identified by all respondents in pastoral and agro-pastoral farming systems (Table 3.2b). Only one smallholder village identified death as a clinical sign of rabies in humans. Ninety three percent of village responses identified coughing as a clinical sign associated with tuberculosis. Skin lesions, diarrhoea and death were identified by 40% village responses as clinical signs of anthrax in humans. Clinical signs associated with brucellosis in humans were malaria-like signs, recurrent fever, joint pains, emaciation and vomiting (Table 3.2b). Clinical signs of FMD were characterised by fever, flu-like symptoms, diarrhoea, headache, coughing and miscarriages with fever, flu-like symptoms and headache being pronounced more in children. Tetanus was characterised by fever and tremors, while worms were associated with segments seen in faeces. Typhus fever was manifested by high fever and general body malaise. Both mange and orf were characterised by skin lesions.

Table 3.2b: Clinical signs associated with zoonoses in humans as identified by respondents during the PRA survey

Disease/signs	***Pastoral villages***	***Agro-pastoral villages***	***Smallholder dairy villages***	*Overall village responses (%)*
Rabies	(n=3)	(n=6)	(n=6)	(N=15)
Madness	3	6	6	**100**
Barking	3	1	1	**33**
Death	3	6	1	**67**
Tuberculosis	(n=2)	(n=6)	(n=6)	(N=14)
Coughing	2	6	6	**100**
Emaciation	1	0	0	**7**
Adenitis	0	1	0	**7**
Anthrax	(n=3)	(n=4)	(n=6)	(N=13)
Skin lesions	2	4	0	**39**
Diarrhoea	2	1	3	**39**
Vomiting	1	0	0	**7**
Death	2	1	3	**39**
Brucellosis	(n=3)	(n=6)	(n=3)	(N=12)
Joint pains	2	0	0	**17**
Recurrent fever	3	2	0	**42**
Vomiting	1	0	0	**8**
Malaria-like	2	4	3	**75**
Emaciation	0	2	0	**17**

3.3.1.1.3 Routes of transmission of zoonotic diseases

Sixty three percent of 19 conditions were reported to be transmitted to humans via ingestion of animal products such as raw milk, meat and blood, whereas 37% of the conditions were considered to occur by direct contact, aerosols and bites. Animal bites were strictly referred to as "dog bites" and reported by 93% village responses as a route of transmitting rabies to humans. No other zoonoses were reported to be transmitted by dog bites.

3.3.1.1.4 Consumption habits of animal products

Boiling of milk for home consumption varied between communities, being reported in 100% smallholder, 80% agro-pastoralist and 0% pastoralist village group responses. One agro-pastoral village responded that many households do not boil soured milk for several reasons, including reduction in butter content and change in flavour. In the smallholder dairy villages, milk destined for sale was left unboiled.

In pastoral communities, blood was consumed when animals were slaughtered at their premises. Furthermore, during famine or when a woman gave birth, blood was obtained by venipuncture from a live animal using an arrow. Villagers only bled healthy animals thought to be free from disease. All village responses indicated that blood was either consumed raw or mixed with hot soup. Agro-pastoralists had three different methods of preparing blood before consumption. The first preparation was raw blood mixed with duodenal content, meat chops and bile (locally known in iraqw as *khansay*). Sixty seven percent of agro-pastoral village responses showed that this preparation is still taken by

some community members. The second preparation was raw blood mixed with hot soup, and the third preparation was fried blood. Fifty percent of village responses admitted consuming one or more of these these preparations. In the smallholder dairy community, 83% village responses showed that blood was fried to make blood pudding. This kind of preparation was currently prepared in some of the local restaurants (locally known as *boms*). Twenty nine percent of village responses stated that blood was cooked and blood meal prepared for animals, including poultry rations. Thirty four percent of village responses indicated that raw blood was fed to dogs.

Meat was cooked in all farming systems. Certain offal such as liver, kidney and lungs were often eaten while still raw in pastoral communities. Meat from cadavers was also eaten as pastoralists claimed that transmission of disease from livestock to humans does not happen once animals have died. Sixty seven percent of the village responses in the pastoralist community claimed that aborted foeti from advanced pregnancies were eaten after cooking whereas others found this distasteful and fed this raw to dogs as a means of disposal. Sixty seven percent of village responses in agro-pastoral communities revealed that aborted foeti were fed to dogs, whereas 33% of village responses stated that foetuses were buried. In the smallholder dairy communities incidences of abortion were rare and when this happened, 83% of village responses stated that aborted foeti were buried.

3.3.1.1.5 Family activities

Five household activities were assessed including milking, herding, assisting calving, handling manure and slaughter. Ninety percent of village responses reported that milking

and manure handling was performed by women in both pastoral and agro-pastoral communities. Except for slaughtering, other activities were performed by family members. In smallholder dairy households however, family members were involved in all livestock related activities. Where the animal owners were employed, and children attended school, an animal attendant was employed to care for the animals. Assisting with calving in smallholder dairy was performed by adult men in the family, or alternatively a nearby livestock extension officer was called to assist, whereas in other systems family members assisted with calving. Slaughtering was done by men in all farming systems, with only few women allowed either to slaughter sick animals or small ruminants in pastoral communities.

3.3.1.2 Cross-sectional questionnaire survey at household level

3.3.1.2.1 Zoonoses

During the cross-sectional survey, individual respondents in 101 households were interviewed in pastoral and agro-pastoral communities. Only one respondent who was from a pastoral household was unaware of health problems associated with keeping livestock in response to the question 'Are you aware of any health risks associated with keeping and eating animal products?' In this study the predominant zoonoses identified during the PRA study namely, tuberculosis, anthrax, rabies and brucellosis were explored in more detail. Tuberculosis, anthrax, brucellosis and rabies were identified as zoonoses by 36%, 48%, 65% and 94% of the respondents respectively (Figure 3.2). There was no statistical significant difference between farming systems in identifying the zoonoses ($p = 0.082$).

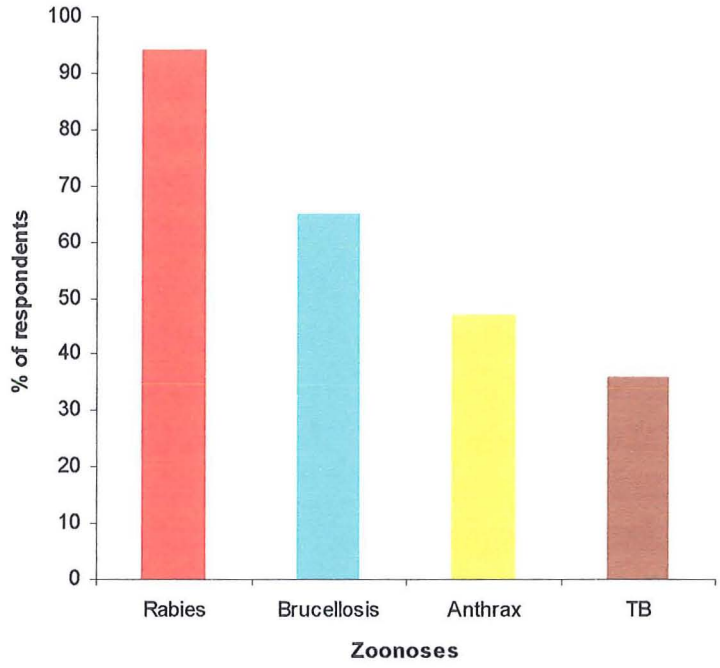


Figure 3.2: Zoonotic diseases identified by respondents

Four domestic species were identified by respondents as involved in contracting the four zoonoses. These species included cattle, sheep, goats and dogs. Cattle, sheep and goats were associated with all four zoonotic diseases identified. Over 90% of respondents identified dogs as contracting rabies while less than 10% identified cattle, sheep and goats as contracting rabies (Figure 3.3). Only 1% of the respondents identified dogs as contracting anthrax. Twenty to sixty percent of the respondents identified sheep, goats and cattle as domestic species that contract brucellosis while 25-45% identified sheep, goats and cattle as contracting anthrax (Figure 3.3). Less than 40% of the respondents identified cattle, goats and sheep as contracting tuberculosis. Agro-pastoral respondents were

significantly knowledgeable of the species associated with zoonoses compared to pastoral respondents ($p = 0.001$).

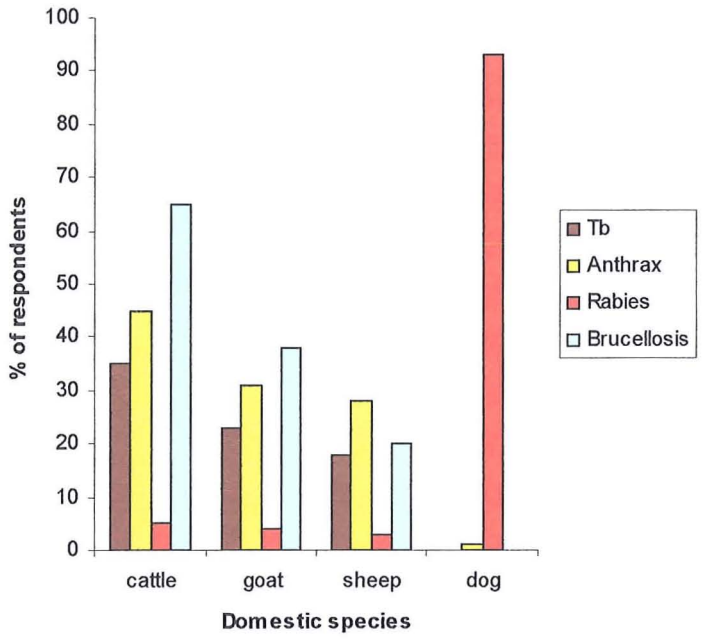


Figure 3.3: Domestic animals associated with zoonoses as identified by respondents during the cross-sectional survey

3.3.1.2.2 Clinical signs of zoonotic diseases in animals and humans

Madness was identified as a clinical sign of rabies in animals by 89% of the respondents. Other clinical signs in animals included barking with a high pitched sound (30%) and abnormal biting (15%), where the animal bites everything possible. Coughing and emaciation were clinical signs identified by 67% and 53% of the respondents as associated with tuberculosis in animals (Table 3.3a). Eighty three percent of the respondents identified

sudden death as a clinical sign associated with anthrax. A drop in milk production, lymphadenitis, swelling of the fore legs and pathological changes such as lack of rigor mortis and blood oozing were identified by less than 10% of the respondents. Abortion, emaciation, a drop in milk production and fever were identified as clinical signs associated with brucellosis by less than 10% of respondents (Table 3.3a). Respondents from agro-pastoral communities were significantly knowledgeable of clinical signs associated with zoonoses in animals compared to pastoral respondents ($p<0.05$).

Table 3.3a: Clinical signs and pathological changes associated with zoonoses in animals as identified by respondents during the cross-sectional survey

Disease/signs	***Pastoral***	***Agro-pastoral***	***Overall)***
Rabies	**(n=27)**	**(n=66)**	**(N=93)**
Madness	21	62	89
Barking	11	17	30
Red eyes	7	2	10
Abnormal Biting	9	5	15
Emaciation	1	3	4
Loss in appetite	0	2	2
Death	1	0	1
Tuberculosis	**(n=4)**	**(n=32)**	**(N=36)**
Coughing	2	22	67
Emaciation	1	18	53
Milk drop	0	5	14
Fever	0	3	8
Death	0	1	3

Anthrax	**(n=14)**	**(n=34)**	**(N=48)**
Sudden death	11	29	83
Milk drop	0	2	4
No *rigor mortis* and blood oozing	0	1	2
Fore legs swelling	0	1	2
Affecting healthy animals	1	0	2
Lymphadenitis	0	1	2
Brucellosis	**(n=6)**	**(n=59)**	**(N=65)**
Abortion	0	1	1.5
Emaciation	0	2	3
Milk drop	1	5	9
Fever	0	2	3

Death, madness and barking were identified by 80%, 70% and 24% of the respondents as clinical signs associated with rabies in humans respectively, while emaciation and salivation were identified by less than 10% of the respondents (Table 3.3b). Coughing and emaciation were identified by 97% and 86% of the respondents as clinical signs associated with tuberculosis, with few respondents noting adenitis, recurrent fever, dyspnoea and haemoptysis, weakness and death (Table 3.3b). Death was identified by 52% of the respondents whereas two to twenty one percent of the respondents identified skin lesions, diarrhoea, vomiting, stomach-ache, high fever and swollen abdomen as clinical signs associated with anthrax (Table 3.3b). Joint pains, headache, recurrent fever and backache were identified by an overall average of 33% of respondents as clinical signs associated with brucellosis (Table 3.3b). Less than 15% of the respondents identified emaciation, body

malaise, loss of appetite and death as clinical signs of brucellosis in humans. The knowledge of clinical signs of zoonoses in humans was not significantly different between pastoral and agro-pastoral respondents ($p>0.05$).

Table 3.3b: Clinical signs associated with zoonoses in humans as identified by respondents during the cross-sectional survey

Disease/signs	*Pastoral*	*Agro-pastoral*	*Overall*
Rabies	**(n=27)**	**(n=66)**	**(N=93)**
Madness	18	47	70
Barking	2	20	24
Salivation	0	4	4
Emaciation	0	1	1
Death	22	52	80
Tuberculosis	**(n=4)**	**(n=32)**	**(N=36)**
Coughing	4	31	97
Emaciation	4	27	86
Adenitis	1	3	11
Recurrent fever	0	4	11
Dyspnoea and haemoptysis	0	2	6
Weakness	0	2	6
Death	1	8	25
Anthrax	**(n=14)**	**(n=34)**	**(N=48)**
Skin lesions	9	1	21

Diarrhoea	3	5	17
Vomiting	0	1	2
Stomach-ache	2	5	15
High fever	5	4	19
Swollen abdomen	0	5	10
Death	7	18	52
Brucellosis	**(n=6)**	**(n=59)**	**(N=65)**
Joint pains	3	22	39
Recurrent fever	2	17	29
Emaciation	0	6	9
Headache	2	22	37
Backache	3	14	26
Body malaise	1	8	14
Loss of appetite	0	5	8
Death	0	3	5

3.3.1.2.3 Routes of transmission of zoonotic diseases

Sixty percent of the respondents reported that tuberculosis, anthrax and brucellosis could be acquired through ingestion of animal products such as raw milk, raw blood and raw meat. Other routes included inhalation for tuberculosis and anthrax, reported by 34 % of the respondents, and contact with animals for brucellosis, rabies and anthrax, reported by 1-5% of respondents. All respondents who mentioned rabies as a zoonoses reported dog bite as the major route of transmitting the disease to humans.

3.3.1.2.4 Consumption habits of animal products

Ninety three percent of respondents from all farming systems boiled fresh milk before drinking. Twenty two percent did not boil milk when it was intended for production of sour milk. Of these, 73% were from pastoral communities and 27% were from agro-pastoral communities. The results showed that 71% of households consumed blood. Of the remaining 29% households, 38% collected blood and gave this raw to dogs, whereas 68% left blood to drain down. The methods of preparation of blood prior to consumption were very variable with raw blood being mixed with hot soup common in 64% of the households (Table 3.4). Although at family level all members consumed blood, men consumed more than others (>60%) (Figure 3.4).

Table 3.4: Different methods of blood preparation in pastoral and agro-pastoral communities

Preparation method/state when consumed	*Pastoral communities (n=26)*	*Agro-pastoral communities (n=46)*	*% of Households (n=72)*
1. Raw blood mixed with hot soup	25	39	88.9
2. Raw blood mixed with milk	1	1	2.8
3. Raw blood mixed with duodenal contents and offals	3	7	13.9
4. Raw blood mixed with ruminal content	2	1	4.2
5. Raw blood	16	5	29.2
6. Raw blood fried	2	29	43.1

Figure 3.4: Consumption of raw blood in pastoral communities

(With permission: Cleaveland, S. 2003)

3.3.1.2.5 Family activities

Of the household activities assessed, milking and manure handling were reported by 78% of respondents as performed by women in both pastoral and agro-pastoral communities. Slaughtering was reported by 77% of respondents as performed by men. Herding was usually done by family members, especially men and children.

3.4 Discussion

This study was carried out to assess and compare knowledge, attitude and practices of livestock keepers on zoonoses in different farming systems. In all farming systems; pastoral, agro-pastoral and smallholder dairy, the most predominant zoonotic diseases reported were rabies, anthrax, tuberculosis and brucellosis; conditions which are potentially devastating through impaired production and reproduction in livestock, and through danger to the health of the human population (Radostits, *et al.*, 2000; Acha and Szyfres, 2001).

Rabies is endemic country-wide in Tanzania (Kilonzo and Komba, 1993) but some epidemic in some pastoral areas surrounding the National Parks (Cleaveland, personal communication, 2005). More than 90% of the respondents in both the PRA and cross-sectional surveys identified rabies as a zoonosis. The fact that a high proportion of livestock keepers identified rabies as a zoonosis compared to other diseases was probably due to knowledge of rabies and fear of contracting the disease, which is likely to have prompted them to report animal bite injuries to hospitals particularly for post-exposure vaccination. Staff at the health facility sometimes stated messages about the risk of rabies such as "vaccinate your dogs," "rabies kills" and "confine your dogs." (Personal observation, 2004). The fact that a high proportion of livestock keepers identified rabies as a zoonosis could be an indicator that the disease is prevalent, although national human rabies cases are greatly under-reported in Tanzania (Cleaveland *et al.*, 2002).

More than 90% of respondents in both the PRA and cross-sectional studies identified dogs as animals associated with rabies. The high proportion of livestock keepers identifying dogs

as animals associated with rabies was consistent with veterinary texts that stated dogs to be the principal domestic animals that are responsible for transmitting rabies to humans and other animals (Radostits *et al.*, 2000). Madness was identified in both the PRA and cross-sectional studies as a major clinical manifestation of rabies in animals, again consistent with veterinary texts (Radostits, *et al.*, 2000; Acha and Szyfres, 2001). Other clinical signs including frequent barking, abnormal biting, red eyes, emaciation, loss of appetite, and death were identified during cross-sectional survey but not in the PRA. These clinical signs are important in the identification of rabid animals. In a comparable PRA study conducted in West Africa dogs, were identified as being associated with rabies with similar clinical manifestations (Unger and Munstermann, 2004).

Madness, barking and death were frequently identified by respondents as associated with rabies in humans in both the PRA and cross-sectional studies. Barking has not however been reported in genuine human rabies cases (Acha and Szyfres, 2001). Identification of barking as a clinical sign of rabies in humans could be due to the fact that rabid patients may produce abnormal sounds following vocal cord partial paralysis. The difference observed between pastoral and agro-pastoral (high response) and smallholder (low response) communities, in identifying death as a clinical sign associated with human rabies may be due to availability of health facilities in smallholder areas compared to pastoral and agro-pastoral areas where such facilities are limited. Also cost implications for post-exposure vaccines may hinder them seeking medical attention.

From 1989 to 2001, 216 outbreaks of anthrax have been reported in livestock in Tanzania affecting 4.5 million cattle (FAO/OIE, 2002). Outbreaks in small ruminants and pigs have

also been reported, albeit at a lower level than cattle. Despite such epidemics in livestock, authentic data on human cases are rare (FAO/OIE, 2002). Several human anthrax cases were reported in Hai district and Dar-es-Salaam following consumption of meat infected with anthrax (Kambarage, 2004). Similar results were reported from Rukwa region and linked with anthrax outbreaks in animals (Webber, 1985). In such outbreaks, 239 human anthrax cases were reported (Webber, 1985). The high proportion of respondents (87%) identifying anthrax as zoonosis during the PRA may indicate a persistent problem in these localities as there were no control measures for anthrax in place and people in pastoral communities are still consuming meat from sick animals and cadavers. Both the PRA and cross-sectional livestock keepers identified domestic ruminants as animals associated with anthrax. This was consistent with veterinary texts that stated domestic ruminants to be responsible for transmitting anthrax to humans (Radostits, *et al.*, 2000; Acha and Szyfres, 2001). The pathological changes and clinical signs of anthrax in animals were consistent with veterinary texts (Radostits, *et al.*, 2000; Acha and Szyfres, 2001). The clinical signs of anthrax in humans reported in both studies were consistent with veterinary texts (Acha and Szyfres, 2001). None of the respondents identified the respiratory form of anthrax in humans, the most important and dangerous form of anthrax in humans as it is always fatal (Guihot, *et al.*, 2004). The disease is acquired through aerosol transmission of anthrax spores. Skinners and those handling infected carcasses could be at risk of acquiring pulmonary form of anthrax in Tanzanian settings.

The fact that a high proportion of livestock keepers that identified tuberculosis as zoonotic was probably due to the increase of tuberculosis cases following the HIV pandemic (Mhalu, 2004). Zoonotic tuberculosis is caused by *Mycobacterium bovis* (*M. bovis*), an agent that

has been reported to occur in Tanzania albeit at low prevalence compared to *M. tuberculosis* (Kazwala *et al.*, 1998). Responses in these studies referred to human tuberculosis in general and thus included infections caused by *M. bovis* and *M. tuberculosis*. Respondents in both the PRA and cross-sectional studies identified domestic ruminants as animals associated with tuberculosis, as in Radostits, *et al.*, (2000). The clinical signs of tuberculosis in animals identified in both the PRA and cross-sectional studies are in conformity with Radostits, *et al.*, (2000). Coughing, emaciation, adenitis and death were the clinical signs of tuberculosis in humans identified by respondents in both the PRA and cross-sectional studies and are consistent with Abram (1985).

Very little is known about human brucellosis, but the fact that a high proportion of livestock keepers in pastoral and agro-pastoral systems identified brucellosis as a zoonosis during the PRA and cross-sectional studies could be an indicator that brucellosis is present in their communities. This has subsequently been confirmed by studies in Northern Tanzania, that show human brucellosis was prevalent and humans probably became aware of the disease after attending health facilities (Kunda *et al.*, 2004). It is noteworthy that more detailed knowledge was shown for brucellosis by respondents in the cross-sectional than in the PRA, both in terms of the species associated with brucellosis and the clinical signs in animals. Where clinical signs were reported (for example abortion, drop in milk production in animals and joint pains, recurrent fever, backache and headache in humans) they were broadly consistent with Radostits, *et al.*, 2000; Acha and Szyfres, 2001). Identifying domestic ruminants as associated with brucellosis is consistent with several studies that showed domestic ruminants are associated with brucellosis in Africa (Mahlau, 1967; Bishop, *et al.*, 1994; Omer *et al.*, 2000b; Refai, 2002).

During the PRA and cross-sectional studies, agro-pastoral communities were shown to be more knowledgeable on the clinical manifestation of zoonoses in animals than other farming communities. This could be due to the fact that the majority of agro-pastoral livestock keepers were pastoralists but evolved into agro-pastoralists following agricultural expansion which resulted in limited land, decreased numbers of livestock for the land to accommodate, and establishment of permanent settlements. This enabled livestock keepers to access basic services such as education and health facilities compared to pastoralists who are either semi-sedentary or nomadic. Formal education to at least primary level education coupled with extension services and messages on disease risks from health facilities were probably an added advantage to agro-pastoral respondents in identifying zonooses and their clinical signs in animals.

The smallholder dairy is a new sector developed from 1980's to meet the demand for milk in urban and peri-urban areas in Tanzania. (Weinhaupl *et. al.*, 2000; Karimuribo, 2002). Therefore, the small proportion of smallholder livestock keepers who identified clinical signs associated with zoonoses during the PRA could be due to the fact they have reared animals for a relatively short time and thus have little knowledge of livestock-derived diseases. These livestock keepers relied on veterinary services rather than dealing with these problems themselves, resulting in poor understanding of these diseases. Although respondents from the pastoral system have kept animals for decades and have knowledge of several livestock diseases, they had little knowledge on clinical signs related to zoonotic diseases. This was probably due to the fact that most of these diseases have no obvious specific clinical signs. Limited knowledge of clinical signs of zoonoses in animals was

reported by respondents during the PRA discussions. This is consistent with veterinary texts where diagnosis of brucellosis and tuberculosis based on clinical grounds is acknowledged as difficult (Radostits, *et al.,* 2000).

From the list of zoonoses in Table 3.1, several conditions did not fulfil the criteria for classification as zoonotic according to WHO definition (1959). Mastitis is a general term referring to udder inflammation. The cause of inflammation could be multifactorial and complex (Karimuribo, 2002). Some causative agents may be zoonotic agents such as *Mycobacterium* and *Brucella species*. In the case of tetanus, Schwabe (1984) and Gracey, (1986) demonstrated *Clostridium tetani* in the intestines of apparently healthy animals such as horses, donkeys and domestic ruminants and the organism is also abundant in faeces. Animal faeces could therefore be a major source of infection to humans through wound contamination (Schwabe, 1984). Consumption of meat with cancer does not transmit disease to humans, but xenotransplantation may pose a threat though this is yet accepted as being zoonotic (Palmer, *et al.,* 1998).

Foot and Mouth Disease (FMD) was reported during the PRA study as a zoonosis in pastoral communities, although FMD was described as not zoonotic by Schrijver *et al.*, (1999). Similar findings were observed in West African countries where FMD was mentioned as zoonosis during the PRA study (Unger and Munstermann, 2004). Recently several reports have shown that there is no doubt that FMD is indeed a zoonosis (Schwabe, 1984; Geoffrey, 1988; Bauer, 1997). However, this has to be differentiated from infections caused by Coxsackie A group, herpes simplex, and sometimes vesicular stomatitis by isolation and typing because they can appear similar clinically (Bauer, 1997; Schrijver *et*

al., 1999). Humans with FMD were thought to show signs related to FMD in livestock including vesicles in the mouth, or on the hands and feet (Bauer, 1997). In contrast, in the study area coughing, fever, flu-like symptoms and miscarriages were reported by respondents. These were not consistent with FMD signs (dryness of the mouth, vesicles in the mouth, lips, tongue, hands, and feet) reported elsewhere (Gracey, 1986; Bauer, 1999). Children showed diarrhoea, flu-like symptoms and high fever. However, the severity of the disease in children reported by respondents during the PRA study was in conformity with Geoffrey (1988), where infection in children was more severe than that observed in adults. Although FMD as a zoonotic agent is still controversial, responses observed in this study suggest that the disease could be a problem in certain communities. Communities that stay in close contact with animals under circumstances of poor hygiene, drinking raw milk even from FMD cases and frequent contact with infected animals could potentially contract infection during outbreaks of FMD in livestock. This may be very different to developed countries where FMD tends to be controlled or eradicated and, if there is outbreak, hygienic measures are undertaken including condemnation of milk where appropriate, controlled movements and use of protective materials while handling such animals (Radostits, *et al.* 2000).

Other interesting findings from both the PRA and cross-sectional studies include failure of respondents to identify other domestic animals as being associated with zoonoses. During the PRA, respondents did not identify small ruminants to be associated with brucellosis. Failure to identify small ruminants as animals associated with brucellosis could expose such communities to *B. melitensis* infection. Pigs were also not associated by livestock keepers with either brucellosis or anthrax. Pigs can transmit *B. suis* to humans through

handling or from contaminated materials (WHO, 1997; Shang, *et al.*, 2002). Pigs were not associated with anthrax in both the PRA and cross-sectional studies although outbreaks of anthrax have been reported in pigs in Tanzania (FAO/OIE, 2002). Pigs were only identified to be associated with *C. cellulosae* during the PRA discussions. In previous studies it was shown that people became aware of *C. cellulosae* following pig traders inspecting the tongue for cysts prior to purchase live pigs (Ngowi, *et al.*, 2001). Although cats were not identified in either the PRA or the cross-sectional studies as animals associated with rabies transmission, several reports have shown them to be associated with rabies, toxoplasmosis and recently Severe Acute Respiratory Syndrome (SARS) (Dunn, 2000; Acha and Szyfres, 2001; Fleck, 2004). Cats should therefore be viewed as animals that can be associated with zoonoses, especially where they are kept as companion animals (WHO, 1997). Furthermore, poultry were not identified to be associated with zoonosis in both the PRA and cross-sectional studies although they are reared in majority of households. Failure to identify poultry as associated with zoonosis was probably attributable to the way the question was asked, and in most cases poultry were not valued like other animals among livestock keepers. Nevertheless poultry have been reported to be associated with emerging zoonoses such as avian flu that cause high economic loss and deaths in humans in Eastern Asia (Fleck, 2004).

In both the PRA and cross-sectional studies it was shown that of all the diseases reported, more than 60% were thought to be transmitted by consumption of animal products. Although meat was usually cooked in all farming systems; milk, milk products, offals and blood were still consumed raw in several households. Similar findings were observed by Gidel *et al.*, (1976); Niwael (2001) and Unger and Munstermann, (2004) where drinking

raw milk and blood was observed among livestock keepers. Although during the PRA and cross-sectional studies the majority of agro-pastoral respondents claimed to boil fresh milk, soured milk is still consumed raw. Selling fresh milk to people at the cow yard is common in rural and peri-urban smallholder dairy areas in Tanzania. Pasteurised milk is only found in urban areas though the supply is not enough to meet the demand. Therefore, more people have access to raw milk than pasteurised milk (Weinhaupl, *et al.*, 2000). Lack of awareness of the risks associated with drinking raw milk, consumer preference for raw milk, and inaccessibility or limited to source of cooking fuels may encourage consumers to drink raw milk.

In the current studies it was shown that different methods were used to prepare blood, but frying is likely to be the only method that renders blood safe for human consumption. Frying however, was observed to only be practiced in some of the agro-pastoral and smallholder households. Consumption of raw blood mixed with soup and other preparations were common practices that may expose consumers to risk of zoonotic infections. Although Niwael (2001) reported no risk associated with drinking blood and brucellosis, such habits could predispose consumers to various diseases and thus should not be ignored. Also the current PRA study revealed that meat from animals which had died and aborted foeti are consumed in pastoral communities. Respondents from agro-pastoral and smallholder systems claimed to bury the cadavers and aborted foeti or to feed them to dogs. Such habits of eating cadavers and aborted foeti as observed in pastoral communities may predispose humans to various zoonoses including, anthrax, tuberculosis and brucellosis. Similar findings were observed in West Africa where animals died of anthrax were eaten after cooking with certain herbs (Unger and Munstermann, 2004). Burying

cadavers and aborted foeti as mentioned by agro-pastoral and smallholder respondents in this study may reduce the risks of zoonotic infections. Feeding aborted foeti to dogs as observed during the PRA study was comparable to Niwael (2001) where 48% of the respondents claimed to feed dogs with aborted foeti. Dogs that fed on aborted foeti are more likely to contract infections such as brucellosis and thus maintain and complicate the infection dynamics in animal and human populations.

In communities where hazard analysis critical control point procedures are well instituted at farm level, food processing plants eg. milk pasteurisation and slaughter houses and during transportation may reduce transmission through ingestion and hence other routes such as contact and inhalation may become important. The means of transmission of zoonoses may therefore vary between communities and countries.

It was observed that during both the PRA and cross-sectional studies the majority of livestock keepers were responsible for all livestock related activities such as slaughtering, milking, hauling manure and assisting with parturitions. Handling such activities under poor hygiene without protective materials could pose serious risks to handlers in addition to consumption of contaminated food (Gracey, 1986; Kumar, *et al.*, 2000). Furthermore Niwael (2001) found that the use of protective materials and disinfectants while handling such cases had never been practiced by livestock keepers though potentially they could reduce risks to handlers considerably.

Both the PRA and structured cross-sectional questionnaire techniques provide consistent findings in this study. Variations were observed in some sections of the findings where the

cross-sectional study yielded more information than the PRA study. For example, clinical signs associated with zoonoses in animals were identified more frequently during the cross-sectional study than the PRA study. Such differences may arise due to the fact that during the cross-sectional study the respondent replied individually to all questions, whereas this was not the case for the PRA that was based on focus group discussions and hence sometimes relied on an agreed group response. A higher proportion of respondents during the PRA study identified the major four zoonoses compared to the cross-sectional study. The difference may be explained by the fact that views expressed by dominant individuals in the group might have taken precedence over other responses and that a group were more likely to suggest multiple responses than any one individual. The PRA therefore is more liable to bias due to lack of randomisation of respondents hence the results may become difficult to be extrapolated or subjected to statistical analysis (Franzel and Crawford, 1989). Despite the potential limitation of these approaches the PRA methodology has been shown to produce reliable and valid information if used with key informants and conducted by experienced persons (Catley, *et al.*, 2002). In addition, during the current study the PRA was shown to have several advantages including, being a rapid tool for baseline data collection where information is not available, involving learning during focus group discussion, useful in nomadic pastoralists, allowing collection of extra information and helping to build relationships between researchers and respondents which creates a favourable environment for future collaboration. Comparable studies conducted in Kenya, Sudan, Gambia, Senegal and Guinea have shown that the PRA technique is useful in data collection (Catley, *et al.*, 2002; Unger and Munstermann , 2004) provided objectives, study population and resources were carefully considered.

CHAPTER IV

4.0 SERO-PREVALENCE OF BRUCELLOSIS IN SMALLHOLDER DAIRY, AGRO-PASTORAL, PASTORAL, BEEF RANCH AND WILD ANIMALS IN TANZANIA

Abstract

A total of 2738 livestock sera from smallholder dairy, agro-pastoral and pastoral herds were screened for antibodies to *Brucella* species using the Rose Bengal Plate test. Screening was also carried out on 403 cattle sera that were purposively collected from one beef ranch which had a history of abortion and also from 90 wild animals that were darted for various purposes. The results revealed a seroprevalence of 6.2%, 6.5% and 13% in cattle, small ruminants and wildlife respectively. Seropositivity based on agro-ecological zones ranged from 0-8%, with seroprevalence significantly higher in the Lake zone than other zones ($p<0.01$). The pastoral farming system had a significantly higher seropositivity ($p<0.01$) than the small holder dairy and agro-pastoral farming systems. Pastoral cattle were three times more likely to be seropositive compared to cattle in the agro-pastoral farming systems

This study shows *Brucella* infection occurs in all farming systems in Tanzania, albeit at variable magnitudes, and is present in both domestic animals and wildlife. Formulation of

strategic control measures to control infection reduce reproductive losses and minimise public health risks are likely to be necessary in many parts of Tanzania.

4.1 Introduction

Brucellosis was first confirmed in Tanzania in 1928 from samples taken from aborted cattle at Engare Nanyuki, Arusha region (Kitaly, 1984). Since then, several surveys have indicated the presence of the disease in all domestic ruminants in various regions and farming systems in different agro-ecological zones. However, interpretation of results has been hindered as a result of variability in sampling and testing methods (Table 4.1).

Table 4.1: The sampling methods and serological tests used to establish seroprevalence of animal brucellosis in different regions of Tanzania

Agro-ecological zones	*Regions*	*Sampling method*	*Test used*	*Prevalence (%)*	*Reference*
Eastern	Morogoro	Convenient and purposive	Indirect ELISA	2-90*$	Minga and Balemba, 1990
	Morogoro	Not known	SAT	5.6#	Swai, 1997
			RBPT	10.6#	
			Indirect ELISA	22#	
	Dar-es-Salaam	Purposive	SAT	14.1#	Weinhaupl, *et al.*, 2000
	Coastal	Convenient	SAT	12.3+	
Central	Dodoma	Purposive	SAT	5.2*#	Kitalyi, 1984

Northern	Arusha	Not known	SAT	6.9+*	Staak and Protz, 1973
	Arusha	Multistage random sampling	IELISA	3.2*	Minja, 2002
Lake	Mwanza	Purposive	SAT	10.8$*	Jiwa *et al.*, 1996
Southern	Mtwara, Lindi & Ruvuma	Not known	SAT	2-13#	Otaru, 1985
Southern Highland	Iringa	Convenient	SAT	4.5#*	Maiseli (1992)
	Mbeya	Convenient	SAT	1.4#	Maiseli (1992)
	Rukwa	Convenient	SAT	6.4*	Maiseli (1992)
	Iringa	Not known	SAT	13.2Δ	Mahlau (1967)

*+ = pastoral herds, * = agro-pastoral herds, # = smallholder dairy herds, $ = beef ranches, Δ = abattoir*

Based on these previous studies several conclusions can be drawn (i) Serological studies indicated that brucellosis was present in all zones with exception of western zone where reports were not available. (ii) All farming systems had reported the infection though limited data were available for pastoral and agro-pastoral farming systems. (iii) Seroprevalence varied between farming systems within zones and between zones. Few studies conducted a survey of more than one farming system and small ruminants were ignored in the majority of the studies even though in the farming systems studied, cattle and small ruminants are herded together.

Disease control measures through vaccination using *Brucella abortus* S19 were instituted only in dairy farms up to the late 1980s, when the programme collapsed due to lack of vaccines and resources (Kambarage 2003, Personal communication). Intervention has been not extended to other farming systems even though seroprevalences were high in some areas. Currently there are no control measures in place in Tanzania although upon screening, voluntary test and slaughter is undertaken by a few livestock owners (Kitaly, 1984) primarily in the smallholder dairy sector.

Brucella seropositivity has also been identified in wildlife species such as impala, topi, buffalo and wildebeest in Tanzania (Schiemann and Staak, 1971; Anderson, 1988; Hamblin, *et al.*, 1990). Recorded seroprevalences were highest in buffalo in Serengeti national park (37%) and Tarangire national park (67%) followed by wildebeest in Serengeti (6.5%) (Anderson, 1988) and lowest in impala (2%) (Schiemann and Staak, 1971). Studies conducted elsewhere indicated that several species of wildlife and marine mammals were infected (Waghela and Karstad, 1986; Madsen and Anderson, 1995; Cheville, *et al.*, 1998; Nielsen *et al.*, 2001; Hillman, 2002). It was observed from these studies that buffalo and wildebeest were most affected among African wildlife animals, whereas bison and elk were most commonly affected wildlife in the USA. The importance of wildlife brucellosis is based on the difficulties in eradication and conflicts between farmers and wildlife experts.

The aim of this study was to exploit sera collected from different species in different regions and farming systems in Tanzania in order to establish the seroprevalence of brucellosis infection and to identify areas for further research.

4.2 Materials and methods

Serum samples were obtained from the serum bank kept at the Sokoine University of Agriculture (SUA), Morogoro and Tanzania National Parks (TANAPA) laboratory Serengeti, Tanzania. Samples were collected from livestock and wildlife between 2000 and 2001. A total of 3048 cattle, 93 small ruminants and 24 wildlife sera were made available for analysis at SUA and 66 wildlife samples were made available and analysed at TANAPA laboratories. The livestock sera were collected from different farming systems in several agro-ecological zones of Tanzania through various projects conducted in each zone (Table 4.2). In the Southern Highland zone, samples were collected by the DFID-funded Mastitis Project, in the eastern zone, by the Tanzania Agricultural Project, in the northern zone, by the DFID-funded Bovine Tuberculosis Project. Livestock sera from the lake zone were collected with assistance from the Project Life Lion whereas, sera from central zone was made available by the Veterinary Investigation Centre (VIC) based at Mpwapwa, in the central zone (Figure 4.1). With the exception of samples from central zone where purposive sampling was used, samples from other zones were collected at random. Samples from the northern and lake zones were collected using multistage sampling of herds and individual animals were selected from the herd by convenience. From the Eastern and Southern Highlands zones, animals were selected using multistage cluster sampling with herd selection criteria based on a maximum of 10 dairy animals. Age and sex of each animal were recorded and made available from few studies.

Wildlife sera were collected opportunistically from the Serengeti-Ngorongoro ecosystem as part of a rinderpest surveillance operation, and made available with the permission of

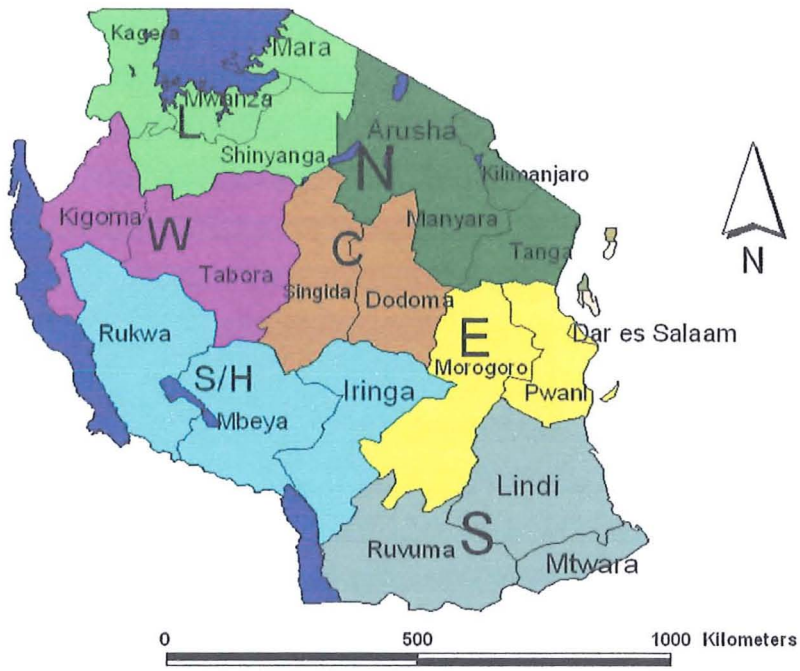
the Chief Veterinary Officer, Tanzania National Parks (TANAPA). The age and sex of these wildlife animals were not available during testing of these samples.

Table 4.2: The distribution of available livestock sera based on agro-ecological zones and farming systems of Tanzania in 2000/2001

Zone	*Projects involved in collection of serum*	*Farming systems*				
		Smallholder	*Agro-pastoral*	*Pastoral*	*Ranch*	*Total*
Southern highland	DFID-funded Mastitis Project	58	0	0	0	58
Eastern	Tanzania Agricultural Project	874	0	0	0	874
Northern	DFID-funded Project Bovine Tuberculosis Project	664	337	165+ 93*	0	1259
Central	Veterinary Investigation Centre (VIC)-Mpwapwa	0	0	0	403	403
Lake	Project Life Lion	0	547	0	0	547
Total		**1596**	**884**	**258**	**403**	**3141**

+Cattle screened from pastoral herds

*Small ruminants screened from pastoral flocks



ZONES: W = Western, L = Lake, N = Northern, C = Central, E= Eastern, S = Southern, S/H = Southern Highland

Figure 4.1 Map of Tanzania showing different zones including zones where livestock sera were collected

4.2.1. Serology

Serology was performed at the laboratory of Sokoine University of Agriculture and the TANAPA laboratory based at Serengeti National Park, Tanzania using a Rose Bengal Plate Test (RBPT) as described in the general methodology (Chapter II section 2.7). These samples were not subjected to c-ELISA as the technique was not in place during this preliminary study.

4.2.2. Statistical analysis

Data were handled using Microsoft Excel Sheet 97 (1993). The 4x2 table was calculated using the Fishers exact test as one cell had zero value (zones and serostatus) and chi-squared test were used to test for associations between seroprevalence and farming systems and age. Ninety five percent confidence intervals were calculated for seroprevalences, using Microsoft Excel.

4.3 Results

Out of 3048 cattle screened, 189 (6%) were seropositive by the RBPT and six out of 93 small ruminants (6.5%) were seropositive using the same test (Table 4.3). Although the proportion of seropositive mature domestic ruminants (those with 3-4 permanent incisors) was higher (11%) than in immature animals (0-2 permanent incisors) (5%) the difference was not statistically significant (χ^2 = 1.2, df =1, 95% CI = 0, 0.139, p>0.05). A higher proportion of seropositive animals was observed in females (8%) than males (6.7%), but the difference was not statistically significant (χ^2 = 0.24, df =1, 95% CI = 0.041, 0.069, p>0.05). The seroprevalence of *Brucella* infection in livestock varied between zones ranging from 0% in the Southern Highlands to 8% in the Lake zone (Table 4.3). There was a significant difference between zones, with a higher cattle seroprevalence recorded in the lake zone (χ^2 = 48.95, df = 3, 95% CI = 0.056, 0.101, p = 0.001) than other zones. The highest seroprevalence of 19% in the Central zone was recorded in the beef ranch which was sampled purposively following a history of abortions.

Seropositivity was significantly higher in pastoral animals (χ^2 = 68.3, df = 2, 95%CI = 0.091, 0.173, p<0.01) than smallholder dairy and agro-pastoral animals. (Table 4.3). Pastoral animals were 2.7 times more likely to be seropositive compared to agro-pastoral animals (OR = 2.7, 95%CI = 1.696, 4.299, p<0.01).

Table 4.3: RBPT seropositivity in livestock under different farming systems from different agro-ecological zones of Tanzania

Zone	*Farming systems*						*Total positives (%)*	*95% CI*
	Smallholder		*Agro-pastoral*		*Pastoral*			
	Tested	*%pos*	*Tested*	*%pos*	*Tested*	*%pos*		
Southern Highlands	58	0	0	0	0	0	0	
Eastern	874	0.8	0	0	0	0	7(0.8)	0.2, 1.4
Northern	664	4.4	337	1.2	165+	17	67(5.3)	4.1, 6.6
					93*	6.5		
Lake	0	0	547	7.9	0	0	43(7.9)	5.6, 10
Total	1596	2.3	884	5.3	258	13.2	195(6.2)	
95% CI	1.5, 2.9		3.8, 6.8		9.1, 17.3			

+ *Cattle*

* *Small ruminants*

Of the 90 wildlife sera obtained from 10 different species, 12 (13%) were seropositive by the RBPT, with seroprevalences of 10% recorded in wildebeest, 28% buffalo and 13% in impala (Table 4.4).

Table 4.4: RBPT seropositivity among wild animals tested in the Ngorongoro-Serengeti ecosystem.

Wildlife	*Samples tested*	*Positive samples n (%)*
Wildebeest (*Connochaetes taurinus*)	29	3 (10.3)
Buffalo (*Syncerus caffer*)	29	8 (27.6)
Baboon (*Cynocephalus ursinus*)	1	0 (0)
Bushbuck (*Tragelaphus scriptus*)	1	0 (0)
Giraffe (*Giraffa camelopardalis*)	5	0 (0)
Topi (*Damaliscus korrigum*)	5	0 (0)
Zebra (*Equus burchelli*)	1	0 (0)
Impala (*Aepyceros melampus*)	8	1 (12.5)
Thomson gazelle (*Gazella thomsoni*)	8	0 (0)
Eland (*Taurotragus oryx*)	3	0 (0)
Overall total	90	12 (13)

4.4 Discussion

The presence of antibodies to *Brucella* in domestic ruminants and wildlife suggests that *Brucella* infection is widespread in both domestic and wild animals in Tanzania, at albeit varying prevalences. The current study showed that there was no significant difference in seropositivity between male and female domestic ruminants. There was no statistically significant difference between cattle age groups. A lack of statistical differences could be due to the fact that such information (age and sex) were not available for the majority of samples during analysis and likely to be confounded by other variables. However, other studies have indicated significantly higher seropositivity in mature animals (Jiwa *et al.*, 1996; Kadohira, *et al.*, 1997; Minja, 2002). As observed by Swai (1997), Weinhaupl, *et al.*, (2000) and Maiseli (1992), the prevalence of brucellosis in smallholder dairy farms in the Southern highland and eastern zones was low as in this study (2.3%) and significantly less than that in traditional and beef animals despite the fact that there is no disease control policy programme, such as the use of S19 vaccination. It is speculated that the characteristics of farming systems such as confinement of animals in houses (zero grazing) and the limited communal grazing which occurs in some urban and peri-urban settings limit the possibility of cross-infection between farms and contamination of communal grazing areas, thereby minimising the establishment of the disease in the sector. Also, introduction of new animals into the herds is limited compared to large herds, due to the nature of management. The small proportion of the seropositive animals however still indicates that infection does occur and calls for concerted efforts in controlling the disease as a large proportion of milk consumed in urban and peri-urban areas is obtained from this sector (Weinhaupl, *et al.*, 2000).

The highest seroprevalence to *Brucella* infection was observed in the Central zone and was expected since this was the result of purposive screening in response to an abortion problem. The seroprevalence in this ranch may not be representative of this type of management system as a whole. Similar studies conducted in ranches in the eastern and lake zones revealed high seroprevalence levels compared to other farming systems (Minga and Balemba, 1990; Jiwa, *et al.*, 1996), however these were also purposive. For example, in the lake zone Jiwa and colleagues (1996) found seroprevalence of 16% in the beef ranches followed by 6.3% in smallholder dairy and lowest (4.3%) in agro-pastoral farming systems. Although risks associated with high seroprevalence in the ranch were not the focus of this study, it is thought that purchase and introduction of new animals from a variety of sources without considering the disease history or prior screening of animals may be a major factor contributing to high levels of infection. The lack of disease control programme and improper disposal of aborted materials may be contributing factors to increased infection rates in the ranch as also observed by others elsewhere (Bishop, *et al.*, 1994; Omer, *et al.*, 2000b; Silva, *et al.*, 2000). Another factor may be herd size (Omer, *et al.*, 2000a; Minja, 2002) as this is likely to increased pasture contamination following calving or abortion. Several studies observed a significantly higher seroprevalence in larger herds (Hellmann *et al.*, 1984; Kadohira, *et al.*, 1997; Omer *et al.*, 2000a; McDermott and Arimi, 2002; Minja, 2002) compared to small herds. This could be explained by the high frequency of introducing new animals into the herds, high levels of interaction within and between herds, and increased movements while seeking pastures, and water especially during dry seasons, and general poor hygiene.

The seroprevalence of *Brucella* infection was significantly higher in pastoral than in agro-pastoral areas. In both farming sectors, animals are kept traditionally and are grazing in communal grazing areas. However, differences between systems exist in that in most agro-pastoral herds, herd sizes are small and the extent of communal grazing is lower than that of pastoral animals. In the pastoral management system, herd sizes are often large; most herds congregate in communal areas and when pastures and water become scarce during the dry period, herds tend to be confined in relatively close proximity in areas where pasture and water are available. In the dry period, relative high stocking rates are not uncommon in the few available places where pastures and water can carry the animals through the dry period. Such congregation allows for increased contamination of pastures and would facilitate cross-infection within and between herds once abortion and deliveries occurred. Movement of animals in search of pasture and water during the dry period also facilitates intermingling with wildlife, and combined use of grazing areas allowing potential transmission of infection between livestock and wild animals. Lack of *Brucella* infection in the Southern Highlands was unexpected as there was no control measures in place. The findings could be attributed to the small number of samples obtained from the zone.

The overall seroprevalence of *Brucella* infection among wildlife species was 13% with a higher infection rate recorded in buffalo (28%) followed by impala (13%) and lowest in wildebeest (10%). The higher seroprevalence observed in buffalo population in this study was comparable to Anderson (1988) who reported seroprevalence of 53% and 37% in buffalo populations in Serengeti and Tarangire national parks respectively. The high levels of infection in buffalo may be one explanation for the declining number of buffalo

population at Ngorongoro-Serengeti ecosystem in recent years (Tanzania Wildlife Conservation and Monitoring, 2004). However, this may need further study and comparison with other areas affected by the disease. Further studies conducted in Kenya and Zimbabwe revealed variable seroprevalences in buffalo which reflects differences in sample size (Waghela and Karstad, 1986; Madsen and Anderson, 1995) and in other species the sample size was too small thus lowering the likelihood of detecting infected animals. In USA bison (*Bison bison*) and elk (*Cervus elaphus*) are the wildlife species reported frequently to have higher seroprevalence of *Brucella* infection especially at Greater Yellowstone Park (OIE, 1997; Cheville, *et al.*, 1998; Hillman, 2002).

The presence of brucellosis in wildlife in Tanzania highlights the potential for wildlife-livestock transmission, particularly in interface areas where wildlife and livestock still co-exist. A similar situation was seen in Yellowstone National Park, USA resulting in major land use conflicts between livestock keepers and wildlife managers (Hillman, 2002). Conversely, livestock may be acting as a source of infection for wildlife with potential threats to the fecundity of ungulate species

As wildlife-based ecotourism is a major source of foreign revenue in Tanzania, there is a need for livestock owners, livestock experts and wildlife conservationists to collaborate and develop appropriate bio-security measures to prevent the spread of brucellosis and other pathogens which are zoonotic. Such collaboration will prevent conflicts that may arise between livestock owners and wildlife personnel that have already been observed elsewhere (Bengis *et al.*, 2002; Thorne, 2004).

It is likely from the results of this study that the entire community is at risk of contracting brucellosis through occupational activities undertaken by farmers, livestock experts, and slaughter personnel and by consumption habits of animal products such as drinking raw milk, blood and undercooked meat. Also wildlife personnel could possibly be at risk especially game officers during cropping (Schiemann and Staak, 1971). Further studies are warranted in areas of high risk such as pastoral and agro-pastoral systems in which the majority of livestock are kept.

CHAPTER V

5.0 A CROSS-SECTIONAL SURVEY IN DOMESTIC RUMINANT AND HUMAN POPULATIONS

Abstract

In 2002 and 2003, a cross-sectional survey was carried out in both domestic ruminant and humans populations in pastoral and agro-pastoral communities of Arusha and Manyara regions, Tanzania to establish the seroprevalence of brucellosis and risk factors associated with seropositivity in domestic ruminants. A competitive enzyme linked-immunosorbent assay (c-ELISA) was used to analyse 3387 livestock sera and 460 human sera in five districts. For the livestock survey, questionnaires were used to collect information at the animal and household level regarding potential risk factors for infection in domestic ruminants.

The overall seroprevalence was 4.7% in cattle, 6.0% in goats and 5.4% in sheep. Seropositivity was detected in cattle and small ruminants in all districts, except Babati district, with the highest seroprevalence (8.6% cattle and 9% small ruminants) recorded in Ngorongoro district. Except for pastoral small ruminants, seroprevalence in both cattle and small ruminants was significantly higher in older animals ($p<0.01$), and in larger herds and flocks ($p<0.01$). In pastoral cattle, but not agro-pastoral cattle, there was a significant

association between c-ELISA seropositivity and retained placenta ($p<0.001$) and between c-ELISA seropositivity and abortion ($p<0.01$) with an attributable risk of 12%.

Brucella seropositivity was recorded in 28% of families, with the highest positive rate in Ngorongoro district (46%) and the lowest in Babati district with no seropositive family. Family members in households with c-ELISA seropositive herds and flocks were 3.3 (OR) times more likely to be c-ELISA seropositive than those with seronegative herds and flocks. However; 25% of c-ELISA seronegative families had c-ELISA seropositive herds and flocks and 48% c-ELISA seropositive families had herds and flocks that were c-ELISA seronegative.

Brucella infection is widespread in domestic ruminants and human populations in northern Tanzania.

5.1 Introduction

Brucellosis is widespread throughout Tanzania and is of concern as a threat to the sustainable economy and food security of rural communities. In livestock the disease causes production losses through abortions, thus reduced replacement animals and, decreased milk production (Radostits, *et al.*, 2000). The disease also results in retained placenta and metritis, which requires costly treatment. Although the disease in cattle was diagnosed in Tanzania in 1928, it was considered by the Veterinary Department as a disease of exotic cattle with little or no economic significance in indigenous herds (Mahlau and Hammond, 1962). After independence in 1961, limited studies were extended to indigenous herds in several regions and seroprevalences varying between 3 to 45%

(Mahlau and Hammond, 1962; Mahlau, 1967; Staak and Protz, 1973; Jiwa, *et al.*, 1996; Weinhaupl, *et al.*, 2000, Niwael, 2001; Minja, 2002). The majority of these studies did not include small ruminants as these were not considered as important as cattle in disease epidemiology, despite the fact that these animals are normally kept together in pastoral and agro-pastoral households. A further limitation of these studies is that few were randomised and they were often carried out purposively in herds with a history of abortions. (Mahlau, 1967, Minga and Balemba, 1990). Furthermore, none of these previous studies have attempted to identify specific risk factors responsible for the transmission of the disease in different farming systems.

Pastoralists depend entirely on livestock and livestock products for their livelihood. Diseases that interfere with livestock production and reproduction have the potential to threaten their livelihood and food security. Therefore, any small improvements in production and reproductive performance are crucial in these communities. This will produce a direct improvement to the family's income and health by having more milk, and indirectly by reducing the human infection burden, therefore saving costs associated with treatment and enabling more people to be involved in productive activities.

In humans, the disease has an acute, subacute and chronic course and the duration varies from few weeks to several months. The predominant symptoms are fever, generalised body malaise, backache, headache and joint pains (Abram, 1985; Acha and Szyfres, 2001). However, these clinical signs are non-specific and the disease can be misdiagnosed and confused with typhoid fever, malaria and rheumatic fever. Humans acquire infection through ingestion, contact, inhalation and accidental inoculation.

Although several countries have carried out studies on human brucellosis (Refai, 2002), few and limited studies have been conducted in Tanzania. Recent studies have shown that human brucellosis was present in some communities in Tanzania (Niwael, 2001; Minja, 2002). There has been no extensive randomised study on human population although livestock studies were extensively conducted in some farming systems.

The objective of this study was to raise awareness about the scale of the problem by:

(a) Establish the magnitude of brucellosis infection in domestic ruminants and humans in pastoral and agro-pastoral communities

(b) Quantify risk factors for infection in livestock

(c) Determine the spatial distribution of brucellosis in the Arusha and Manyara regions.

5.2 Materials and methods

5.2.1 Study area

The study area and farming systems have been described in detail in Chapter II section 2.1.

5.2.2 Study villages and animal sampling

The cross-sectional study was carried out from May 2002 to July 2003. Livestock-keeping households were selected by a process of multi-stage random sampling. The sampling frame comprised a list of all villages in the study area (n=285), which was made available

at district livestock offices. A random sample of 32 villages was selected using a table of random numbers. Among these, 20 were agro-pastoral and 12 pastoral villages. In each village multistage sampling was used to select at random two sub-village administrative units, (known as *kitongoji*). A ten-cell leader (*balozi*), a leader of ten or more households, was selected at random from each sub-village and all livestock-keeping households were identified. Finally, two livestock-keeping households were randomly selected from each ten-cell unit. The initial sampling procedure (involving 12 villages) involved selection of two households from one balozi within each village. This achieved a wide geographic coverage but was considered to be too time-consuming and the sampling procedure was therefore revised to include two households from each of two ten cell units as illustrated in Fig. 5.1.

A required sample size of 2000 cattle and 1500 small ruminants was estimated, which would be sufficient to detect a seroprevalence of 5% (a figure that was considered likely on the basis of previous published studies) with 80% power and 95% confidence. The sample size was calculated as described by Martin *et al.*, (1987) to obtain the total number of animals to be screened from each selected household.

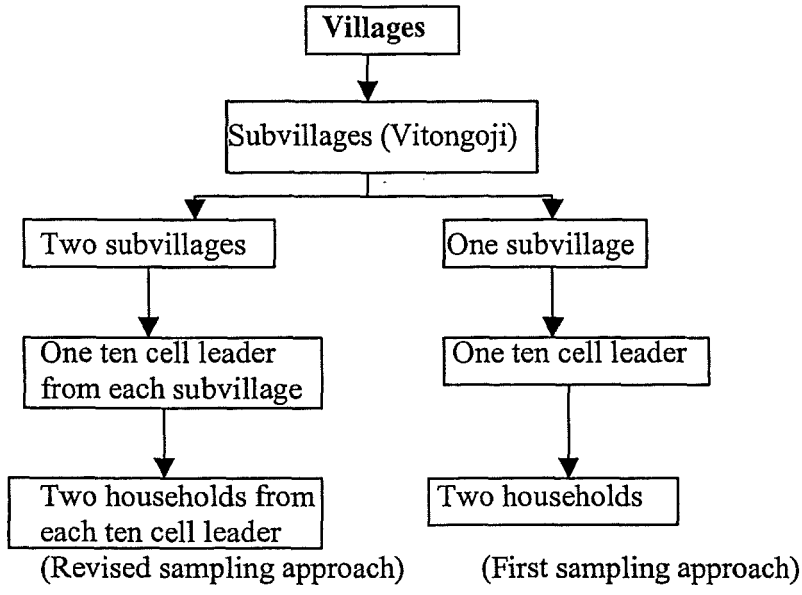


Figure 5.1: cross-sectional household sampling

5.2.3 Livestock and Human sampling

5.2.3.1 Livestock data and sample collection

Following household selection, an initial visit was made to arrange the forthcoming activities with the herd owner. On the day of the visit, all animals in the household were collected in a central location, usually an enclosure (boma) surrounded by a thorn fence. In the absence of any crush facilities, animals were restrained manually, often by roping one of the hind limbs, or holding the horns, tail or forelimbs. In some cases, the animal was cast and restrained in lateral or sternal recumbency. Blood samples were collected from the jugular vein using a sterile needle and a plain vacutainer (Becton and Dickinson, UK) and the metal tag (Ketchum, UK) was fitted to each animal for subsequent identification. Despite preliminary attempts to sample animals at random within the herd, this was difficult to achieve without a systematic method of restraint, such as a crush or race. For

most of the herds, blood samples were collected from any animals restrained by the householders without systematic or truly random selection of individuals.

Each blood sample was labelled using the tag number assigned to the individual animal. Individual animal information was obtained from the herd owner by personal questionnaire at the time of sampling. The information collected included history of abortion, retained placenta, the past two calving dates and current reproductive status. The dentition and sex of each animal was recorded. Age of the animals was determined by the number of permanent incisor pairs present. The age of the animal was recorded as zero if there were no permanent incisor pairs, and 1, 2, 3 or 4 according to the number of permanent incisor pairs respectively.

5.2.3.2 Human data and sample collection

Permission to collect human serum samples was obtained following approval by the ethics committee of the National Institute of Medical Research (NIMR), from the Ministry of Health, Tanzania. In each livestock-keeping household, family members were approached to identify volunteers for blood sampling following discussions about the purpose of the project and the nature of the brucellosis problem. Where householders gave consent, blood samples were collected from the brachial vein after disinfection using cotton wool soaked in methylated spirit (Bell Chemicals Co. Ltd. Dar es Salaam). Blood was aseptically collected using a disposable 5ml syringe (Young Wood Co-operation, Korea). The blood was immediately transferred into a plain vacutainer, assigned an identification number, and

kept in a tray for serum separation. In 14 households, no family member was bled because of lack of consent or because families were far from the livestock enclosures.

5.2.4 Risk factors associated with brucellosis in livestock

The questionnaire was informally pre-tested on veterinary colleagues to detect possible ambiguities or defects in design. Field-testing was then conducted in pastoral and smallholder dairy households in Monduli district, Arusha region before the final version was developed. The livestock questionnaire was designed to obtain information on a wide range of topics including herd management practices, knowledge and awareness of livestock brucellosis, livestock movements and interactions with wildlife animals (Appendix I). The information collected included retrospective data from the previous 12 months. An interview took about 30-40 minutes.

The livestock questionnaire survey was conducted by a single interviewer (author) in 104 households and carried out after bleeding livestock and humans. The interview involved one family member who was knowledgeable about the herd and flock, usually the head of the household. The geographic location of each household was recorded using a hand-held Garmin® Global Positioning Systems (GPS) and recorded as latitude and longitude.

5.2.5 Serology procedures

The Rose Bengal plate test (RBPT) antigen used in the study was *Brucella abortus* Rose Bengal-stained antigen kindly donated by Veterinary Laboratory Agency (VLA)

Weybridge, UK (batch numbers 269 and SG276). The field RBPT was done as described in Chapter II section 2.7.1. In the field the test analysed 3561 livestock sera and feedback was given to respective household owners. During the study, 476 human sera were collected and analysed in the field using RBPT. The results were reported back to family members on the same day and any person who had positive result was asked for any symptoms they had. Individuals were given a written note and advised to seek medical attention immediately for further evaluation. All livestock and human samples were sent to VLA, Weybridge for c-ELISA analysis as a confirmatory test. The detailed procedure was described in Chapter II section 2.7.2. Therefore, a household (with cattle and small ruminants) and a family were considered c-ELISA seropositive if at least one individual was seropositive.

5.2.6 Data storage and analysis

Data were entered using Microsoft Excel spread sheet 97 (1993). The Chi-square test was used to compare two or more proportions and to determine associations. The Fisher exact test p-value was adopted when one or more of the expected cell values was less than 5. The strength of the association between risk factor and brucellosis status was examined by odds ratio (OR) and 95% confidence intervals (95%CI) values. OR was estimated as the ratio of the odds of disease in exposed individuals to the odds in those unexposed (Thrusfield, 1995). The attributable risk was calculated as the difference between the incidence of disease in exposed animals and the incidence in unexposed animals **[a/(a+b)]-[c/(c+d)].**

Multivariate analyses were carried out using generalised linear mixed effects models, with binary outcome of the cross-sectional data at the animal level fitted using EGRET for Windows software (Gogte, *et al.*, 1999). Animal and farm level datasets were merged before copying them into the EGRET programme. Logistic regression with a random effect model was then fitted to assess household effects in the study area. Data analysis was performed by fitting a logistic binomial regression for distinguishable data using the modified Newton Raphson algorithm procedure (Gogte, et al., 1999). The association between c-ELISA results and animal and household level explanatory variables were analysed initially using univariable binomial regression model.

A final multivariate model was fitted using a forward stepwise procedure. Variables with likelihood statistic ratio of less than 0.2 were selected for multivariate analysis.

5.3 Results

5.3.1 Domestic ruminant cross-sectional survey

5.3.1.1 Individual domestic ruminant serology

A total of 3561 domestic ruminants (cattle, goats and sheep) were bled for brucellosis screening from the study area. Of these, 174 samples were not tested. C-ELISA seropositivity was detected in all species although the difference between cattle (4.7%) and

small ruminants (5.8%) was not statistically significant (χ^2= 1.95, df = 1, p>0.05) (Table 5.1).

Table 5.1: Domestic ruminants sampled and sera tested using c-ELISA

Domestic ruminants	*Samples collected*	*c-ELISA tested*	*Positive samples*	*%positive*	*95%CI*
Cattle	1808	1714	81	4.7	0.037, 0.057
Small ruminants	1753	1673	97	5.8	0.047, 0.069
Total	3561	3387	178	5.3	0.004, 0.028

C-ELISA seropositivity increased with age. Domestic ruminants with no permanent incisors had the lowest seroprevalence compared to older animals (Figure 5.2) The difference in c-ELISA seropositivity between young (0-2 permanent incisor pairs) and older (3-4 permanent incisor pairs) domestic ruminants was statistically significant (χ^2 = 25.18, df = 1, 95% CI 0.0258, 0.0538, p<0.01). A similar significant difference was observed in cattle (χ^2 = 9.6, df = 1, 95%CI = 0.012, 0.052, p<0.05) and small ruminants (χ^2 = 9.6, df = 1, 95%CI = 0.012, 0.052, p<0.05) where older animals had more c-ELISA seropositivity than young animals.

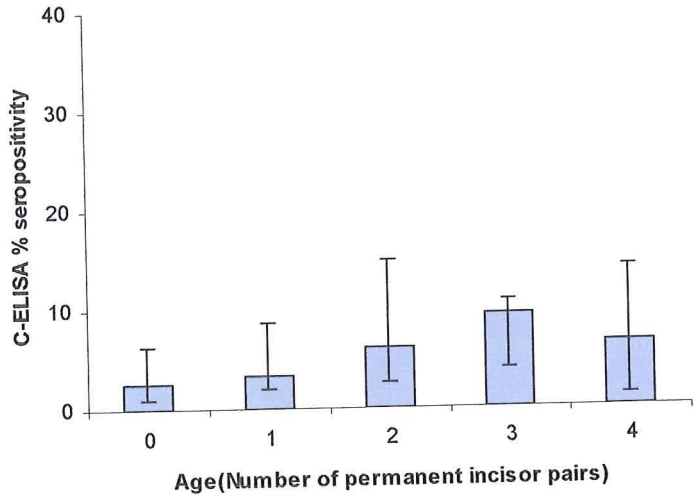


Figure 5.2: Relationship between age and c-ELISA seropositivity in domestic ruminants

A total of 171 (37%) female cattle had calving interval greater than 12 months (mean 16.3 months). Of these 5.3% were c-ELISA positive. In small ruminants 4 (0.6%) animals had parturition intervals greater than 12 months (mean 12.5 months). Of these only one animal was c-ELISA seropositive. There was no significant association observed between calving intervals and c-ELISA seropositivity (95%CI 0.470, 1.805, p=0.8096) though high seroprevalence was observed in animals with long calving interval (Figure 5.3).

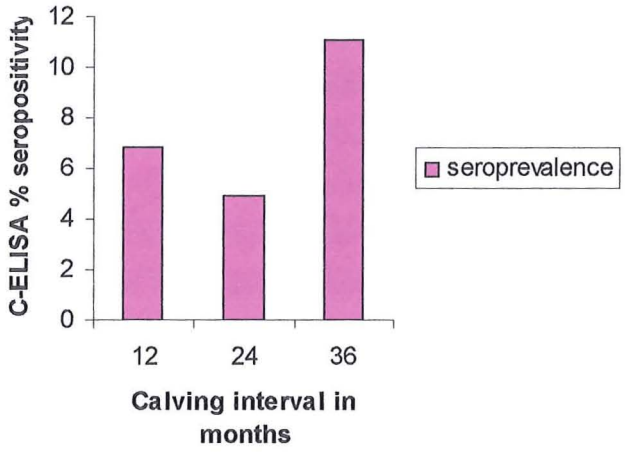


Figure 5.3: Relationship between calving interval and c-ELISA seropositivity in cattle

Out of 2236, 288 (12.9%) female domestic ruminants had a history of abortion and 10.7% of these were c-ELISA positive. A significant association was demonstrated between c-ELISA seropositive domestic ruminants and a history of abortion (OR = 2.1, 95% CI= 1.745, 3.199, $p<0.01$). Among cattle that had a history of abortion, 15.1% (14/93) were c-ELISA positive, whereas in small ruminants 8.7% (17/195) of those with a history of abortion were c-ELISA seropositive. There was a significant association (OR = 3.6, 95%CI = 3.24, 6.83, $p<0.01$) between cattle that had aborted with c-ELISA seropositivity whereas, no statistical association was demonstrated between aborted small ruminants and c-ELISA seropositivity ($p>0.05$). In cattle the attributable risk was 12%.

A total of 37 female cattle had history of retained placenta. Of these 10.8% were c-ELISA positive. There was no significant association between cattle with retained placentae and c-ELISA seropositivity (Fisher's exact p=0.123). No female small ruminant had a history of retained placenta with c-ELISA seropositivity.

5.3.1.2 Herd and flock c-ELISA positivity between and within different farming system

A significantly higher percentage of cattle were seropositive in the pastoral farming system (7.3%) than the agro-pastoral farming system (1.1%) (χ^2 =35, df = 1, 95%CI = 0.044, 0.080, p<0.01). A significant difference was observed between small ruminant c-ELISA seropositivity in pastoral and agro-pastoral systems (χ^2 =48, df = 1, 95%CI = 0.06, 0.010, p<0.01) where pastoral small ruminants had higher c-ELISA serpositivity (9.2%) compared to agro-pastoral small ruminants (1.2%). The proportion of herds and flocks containing at least one seropositive animal is shown in Table 5.2 in relation to size of herd or flock and farming systems. Of 104 households screened, 40 (37.7%) households were c-ELISA seropositive. During the study, 102 herds of cattle were tested and 25 (24.5%) herds were c-ELISA seropositive. Small ruminants were screened from 89 flocks and 26 (29.2%) were c-ELISA seropositive.

5.2: Herd and flock sero-prevalence according to herd/flock size and farming systems

Herd/flock size	*Pastoral herds*		*Pastoral flocks*		*Agro-pastoral herds*		*Agro-pastoral flocks*	
	Screened	*Positive n(%)*	*Screened*	*Positive n(%)*	*Screened*	*Positive n(%)*	*Screened*	*Positive n(%)*
0-20	3	1 (33.3)	0	0 (0)	64	4 (6.3)	40	4 (10)
21-40	6	1 (17)	2	2 (100)	5	0 (0)	17	4 (23.5)
41-60	3	3 (100)	8	3 (37.5)	2	1 (50)	5	1 (20)
61-80	5	3 (60)	4	2 (50)	1	0	1	0
81-100	2	2 (100)	2	2 (100)	0	0	0	0
101-120	5	5 (100)	1	0 (0)	0	0	0	0
121-140	1	1 (100)	1	0 (0)	0	0	0	0
141-160	1	0 (0)	4	4 (100)	0	0	0	0
161-180	1	1 (100)	1	1 (100	0	0	0	0
181-200	0	0 (0)	1	1 (100	0	0	0	0
281-300	1	1 (100)	1	1 (100	0	0	0	0
>300	2	2 (100)	1	1 (100)	0	0	0	0
Total	30	20(67)	26	17(65.4)	72	5(7)	63	9 (14.3)

For both herds and flocks, c-ELISA seropositivity was significantly higher in pastoral than in agro-pastoral farming systems. The c-ELISA seropositivity in pastoral herds was high at 67% when compared to agro-pastoral herds where only 7% were positive (χ^2 = 55.94, df =1, 95%CI = 0.419, 0.776, p<0.01). A similarly significant different was observed in pastoral flocks (χ^2 = 23, df = 1, 95%CI = 0.309, 0.713, p<0.01).

Fifty four percent of the household (herds and flocks) that were c-ELISA positive had a history of abortion. There was a significant association between c-ELISA positive households and a history of abortion (OR = 3.5, 95% CI = 0.001, 8.02, p<0.01). Among pastoral households, 83.3% had a history of abortion compared to 25% of households in agro-pastoral system. Pastoral households were 15 times more likely experience abortion compared to agro-pastoral households, a difference that was statistically significant (χ^2= 16.4, df = 1, 95% CI= 0.351, 0.809, p<0.01).

5.3.1.2.1 Pastoral farming system

The number of animals sampled in the pastoral farming system is reported in Table 5.3. Cattle were drawn from 30 herds and 20 (67%) were c-ELISA positive. Out of 26 flocks screened, 17 (65%) were c-ELISA positive. C-ELISA seropositivity was highest in goats (9.7%) followed by sheep (8.3%) and cattle (7.3%). The difference between cattle and small ruminant seropositivity was not statistically different (χ^2= 3.1, df = 1, 95%CI = 0.003, 0.005, p>0.05). C-ELISA seropositivity was higher in female cattle (7.6%) than male cattle (6.5%). There was no statistically significant difference between male and female cattle c-ELISA seropositivity (χ^2= 0.4, df = 1, 95%CI = 0.023, 0.046, p>0.05). Also

C-ELISA seropositivity was higher in female small ruminants (10%) than males (7.2%). However the difference was not significantly different (χ^2= 1.7, df = 1, 95%CI = 0.011, 0.067, p>0.05).

Table 5.3: Domestic ruminants screened for brucellosis in the pastoral farming system.

Domestic ruminants	*Total animals screened*	*Number of herds/flocks*	*Number of positive domestic ruminants, n (%)*	*Positive herds/flocks, n (%)*
Cattle	997	30	73 (7.3)	20(67)
Goat	648	26	63 (9.7)	17(65)
Sheep	303		25 (8.3)	
Total	1948		161 (8.3)	

A history of abortion was described in 207 domestic ruminants and 18% of these were c-ELISA positive. There was a significant association between domestic ruminants with a history of abortion and c-ELISA seropositivity (OR = 2.5, 95%CI = 1.653, 3.781, p<0.01). There was statistical association between cattle and small ruminant abortion with cattle abortion being 5 times more likely in c-ELISA seropositive animals (OR = 4.85, 95%CI = 0.157, 0.374, p<0.01) and small ruminant abortion being 2 times more likely in c-ELISA seropositive animals (OR = 1.6, 95%CI 0.086, 0.202, p<0.05). Out of 31 pastoral households 77% had history of abortion in domestic ruminants. Of these, 83% were c-ELISA seropositive. There was no statistical association observed between household abortion and c-ELISA seropositivity (Fisher's exact p = 0.338). Of 37 individual domestic ruminants with history of retained placenta, 12% were c-ELISA positive. A statistical association was observed in cattle that had a history of abortion and c-ELISA

seropositivity (OR = 7.9, df = 1, 95%CI = 2.2, 29.0, p<0.01). No small ruminant had a history of abortion with c-ELISA seropositivity. Fourteen households had a history of retained placenta and of these 93% were c-ELISA positive. Households with a history of retained placenta were statistically associated with c-ELISA seropositivity (OR = 58.5, 95%CI = 7.058, 484.856 Fisher's exact test p< 0.001).

C-ELISA seropositivity was higher in larger herds and flocks (Figure 5.4). There was statistical significant difference between c-ELISA seropositivity and age of cattle with high seropositivity observed in adult animals (χ^2= 9.4, df = 1, 95%CI = 0.018, 0.084, p<0.01). There was no statistically significant difference between c-ELISA seropositivity and age of small ruminants (χ^2= 1.4, df = 1, 95%CI = 0.014, 0.060, p>0.05).

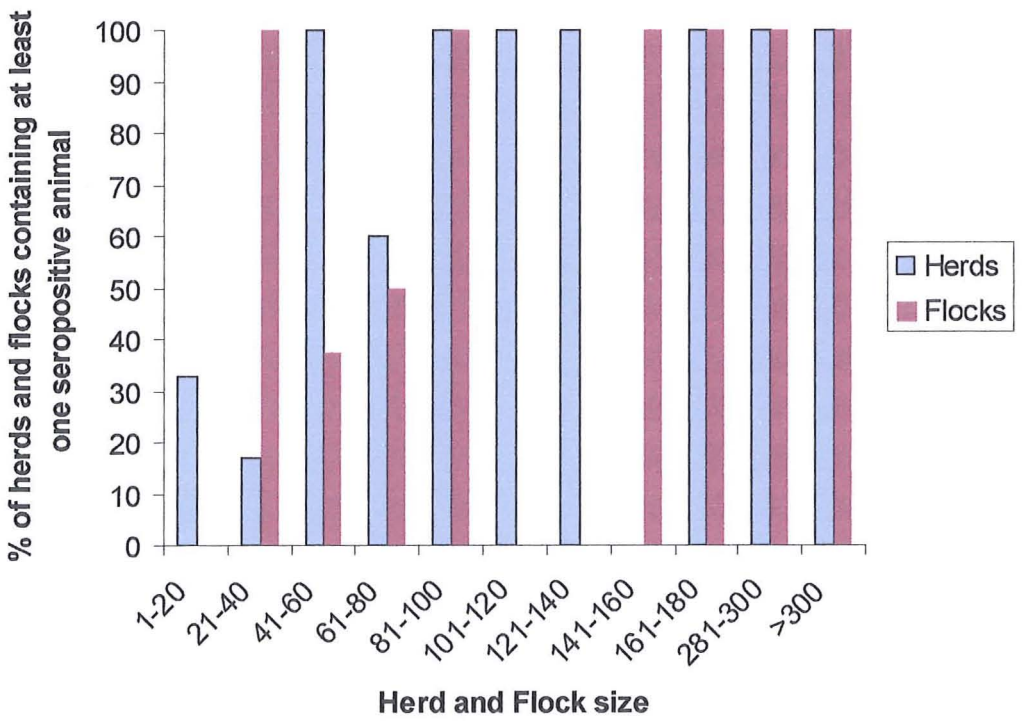


Figure 5.4: Relationship between herd and flock sizes and c-ELISA seropositivity in pastoral systems

5.3.1.2.2 Agro-pastoral farming system

In the agro-pastoral farming system, 1439 domestic ruminants were tested. Cattle were screened from 72 herds and 5 (7%) were c-ELISA positive, whereas small ruminants were drawn from 63 flocks and 9 (14.3%) flocks were c-ELISA positive. Goats had higher c-ELISA seropositivity (1.5%) followed by cattle (1.1%) and lowest in sheep (0.6%) (Table 5.4). There was no significant difference between cattle and small ruminant seropositivity (χ^2= 2.2, df = 1, 95%CI = 0.0026, 0.0189, p>0.05). C-ELISA seropositivity was higher in female cattle (7.6%) compare to male cattle (6.5%) but the difference was not statistically significant (χ^2= 0.38, df = 1, 95%CI = 0.023, 0.046, p>0.05). Similarly c-ELISA seropositivity was higher in small ruminant females (1.5%) compared to males (0.5%) but the difference was not statistically significant (χ^2= 1.3, df = 1, 95%CI = 0.004, 0.025, p>0.05).

No individual domestic ruminants among those screened had a history of abortion with c-ELISA seropositivity. Twenty four households had a history of abortion and 25% were c-ELISA positive. There was no statistical association between history of abortion at herd or flock level with c-ELISA seropositivity (Fisher's exact test p = 0.21). There was no individual domestic ruminant or household with a history of retained placenta and being c-ELISA seropositive.

Table 5.4: Domestic ruminants screened for brucellosis in the agro-pastoral farming system.

Domestic ruminants	*Total screened*	*Number of herds/flocks*	*Number of Positive domestic ruminants n (%)*	*Positive herds/flocks n(%)*
Cattle	717	72	8 (1.1)	**5(7)**
Goats	541	63	8 (1.5)	**9(14.3)**
Sheep	181		1 (0.6)	
Total	**1439**		**17 (1.2)**	

There was statistically significant difference between c-ELISA seropositivity and age of cattle with high seropositivity observed in adult animals (χ^2= 3.96, df = 1, 95%CI = 0.001, 0.03, $p<0.05$). A higher proportion of adult small ruminants were c-ELISA positive than immature animals (χ^2 = 5, df = 1, 95% CI = 0.003, 0.034, $p<0.05$). Although flock seroprevalence appeared to increase with size (Figure 5.5) the difference between c-ELISA seropositivity and flock size was not statistically significant (Fisher's exact test p = 0.055). The difference was however, statistically significant (Fisher's exact test p = 0.0035) in herds where c-ELISA seropositivity was high in big herds.

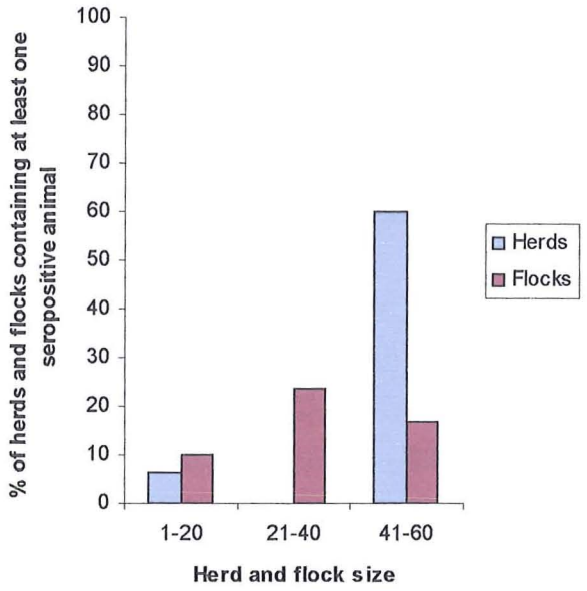


Figure 5.5: Relationship between herd and flock sizes and c-ELISA seropositivity in agro-pastoral systems

5.3.1.3 Spatial distribution of herds and flocks

The spatial distribution of herds and flocks c-ELISA seropositivity is shown in Figures 5.6 a, b c. C-ELISA seropositivity in both herds and flocks was high in pastoral households in Ngorongoro district compared to agro-pastoral households in Karatu, Mbulu, Hanang and Babati districts. (Table 5.5). It is worth noting that several households did not keep sheep and none of the households were positive in Babati district. The mean number of domestic ruminants was highest in pastoral households (73 per household) and lowest in agro-pastoral households (15 per household). C-ELISA seropositivity in pastoral households varied from 0.98 to 29.8% with a mean of 8.7% and variance of 64.3% whereas, in agro-pastoral households, this varied from 1.3 to 14.3% with a mean of 4.3% and variance of 19.1%.

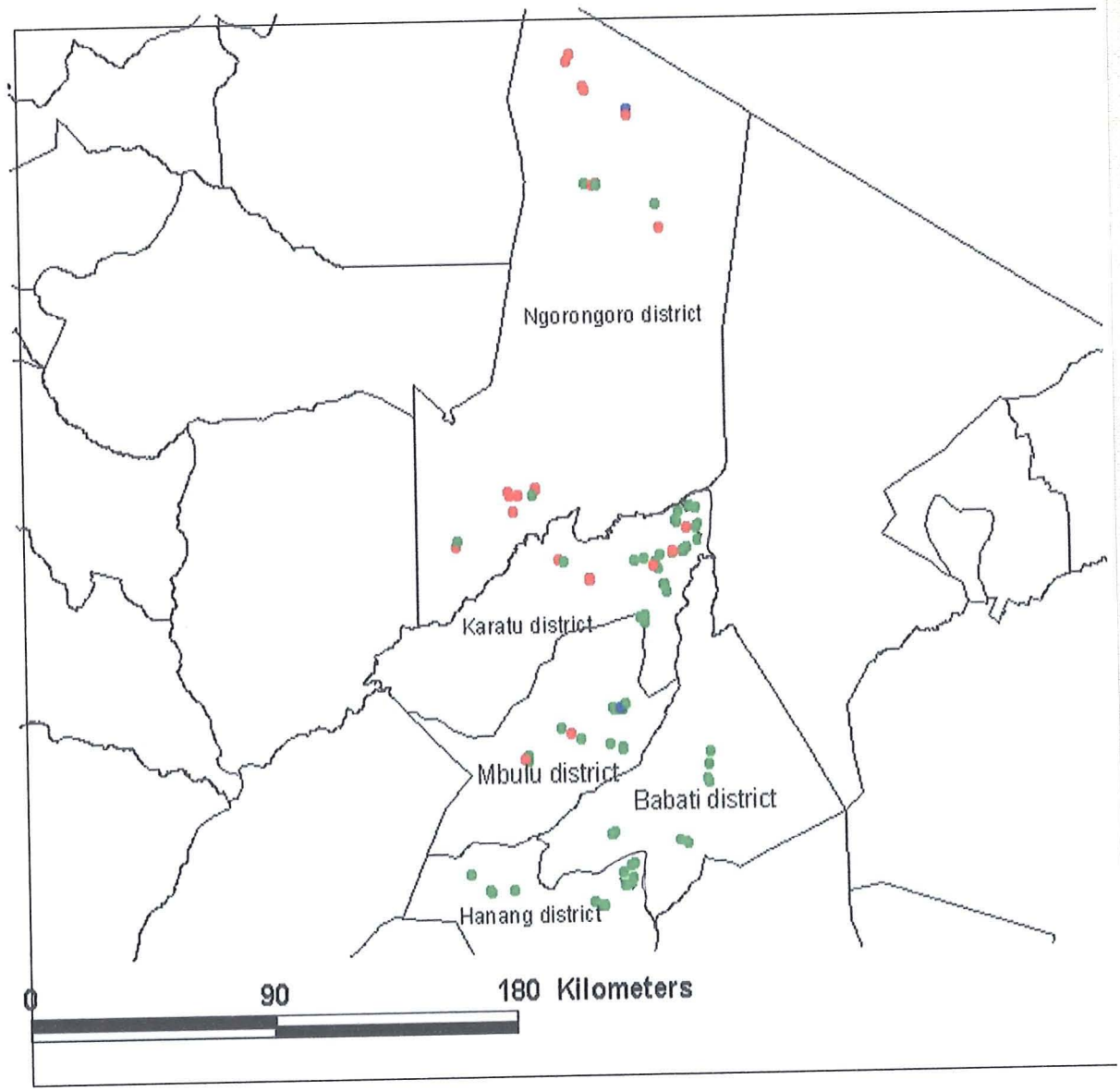


Figure 5.6 (a): Spatial distribution of positive cattle herds in the study area in 2003

Circles: Red = positive herds, Green = Negative herds, Blue = No cattle

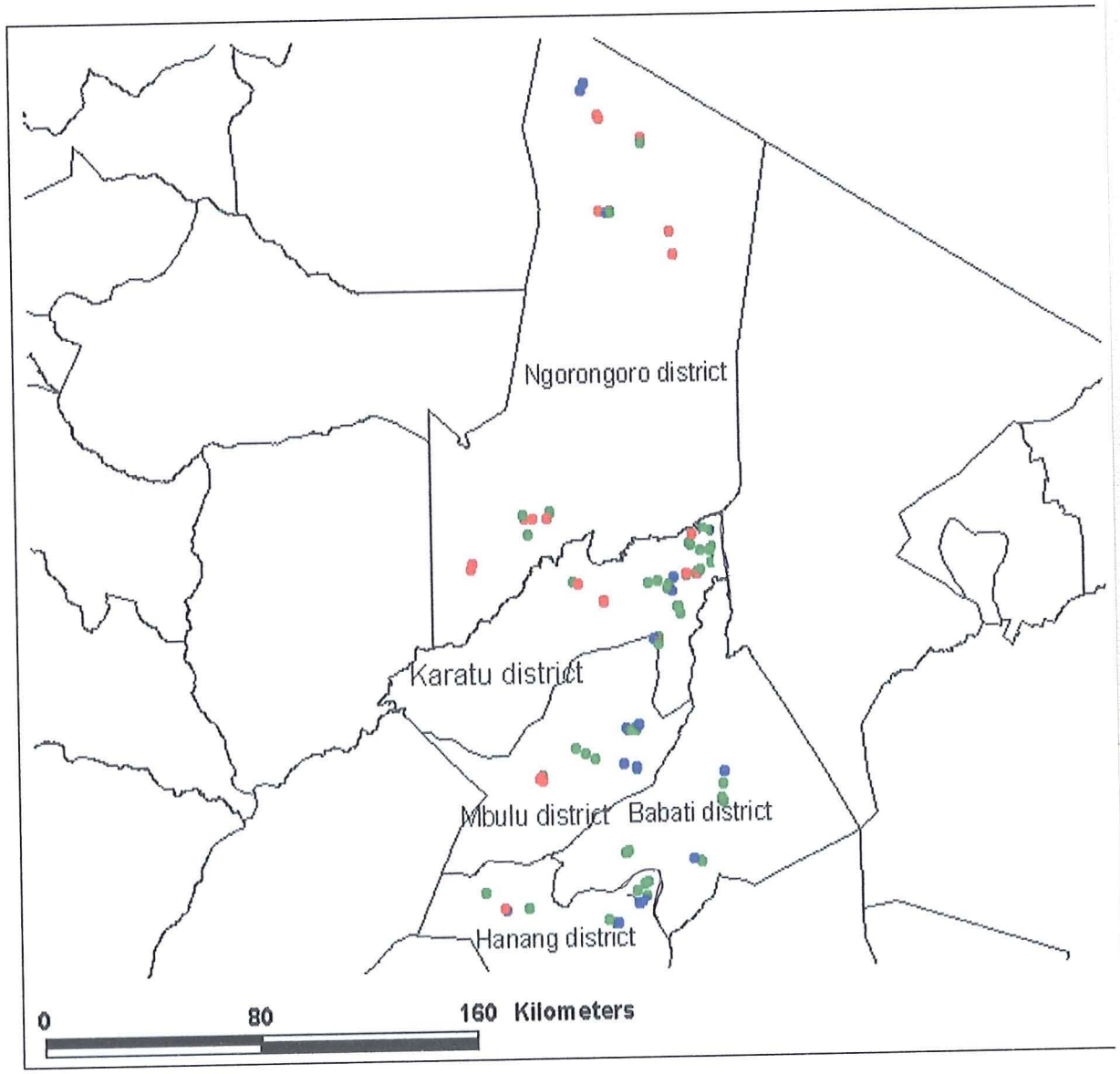


Figure 5.6 (b): Spatial distribution of positive goat flocks in the study area in 2003

Circles: Red = positive flocks, Green = Negative flocks, Blue = No goats

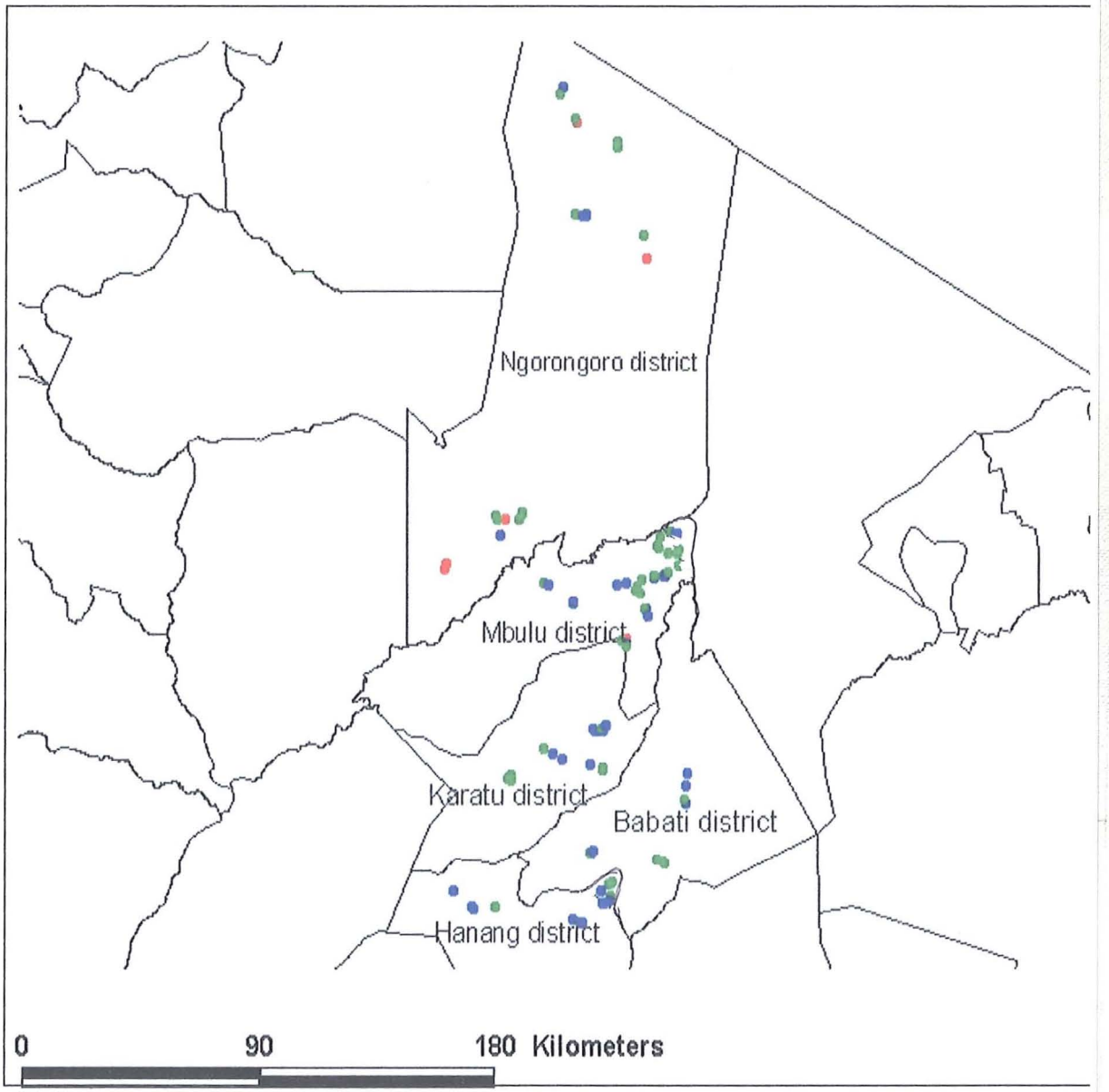


Figure 5.6 (c): Spatial distribution of positive sheep flocks in the study area in 2003

Circles: Red = positive flocks, Green = negative flocks, Blue = No sheep

Table 5.5: Distribution of c-ELISA positive animals and households by districts

District	*Herds*	*Cattle*	*Positive herds n(%)*	*Positive cattle n(%)*	*Flocks*	*Small ruminants*	*Positive flocks n(%)*	*Positive small ruminants n(%)*
Babati	12	100	0(0)	0(0)	11	82	0(0)	0(0)
Hanang	14	190	0(0)	0(0)	10	129	1(10)	3(2.3)
Karatu	38	480	6(16)	10(2)	37	435	9(24)	16(3.7)
Mbulu	17	137	2(12)	2(1.5)	13	222	3(23)	4(1.8)
Ngorongoro	21	807	17(81)	69(8.6)	18	805	13(72)	74(9)
Total	102	1714	25(25)	81(4.7)	89	1673	26(29)	97(5.8)

5.3.1.4 Risk factors for brucellosis in domestic ruminants

Distribution and results of univariate analysis of risk factors and c-ELISA seropositivity are shown in Tables 5.6a and 5.6b for domestic ruminant level predictor variables. Significant risk factors at the domestic ruminant level included: (i) Brought in domestic ruminants (ii) Domestic ruminant abortions (iii) Animal age. Brought in domestic ruminants were 1.2 (OR) times more likely to be c-ELISA positive compared to homebred domestic ruminants. Mature domestic ruminants with 3 to 4 permanent incisors were 3 (OR) times more likely to be c-ELISA positive compared with young domestic ruminants (Those having permanent incisor pairs from 0-2). Variables that were not significantly associated with c-ELISA seropositivity at the animal level were animal type (cattle, goats and sheep), sex, and reproductive status of the animal (Pregnant, Not pregnant, Castrated, Not castrated, Less than 2 months post-calving and calves less than 6 months of age) (Table 5.6b).

Table 5.6 (a): Distribution of c-ELISA positives by animal level variables.

Variable	*Domestic ruminants tested*	*Domestic ruminants c-ELISA positive*	*%positive*
1. Domestic ruminants			
a. Cattle	1714	81	4.7
b. Goats	1189	71	6.0
c. Sheep	484	26	5.4
2. Domestic ruminant source			
a. Homebred	2936	155	5.3
b. Brought in	311	15	4.8
3. Sex			
a. Female	2369	138	5.8
b. Male	1018	40	3.9
4. Age by dentition			
a. 0	932	23	2.5
b. 1	331	15	4.5
c. 2	311	18	5.8
d. 3	212	18	8.5
e. 4	1601	104	6.5
5. Domestic ruminant abortion			
a. Yes	289	27	9.3
b. No	1949	104	5.3
c. Not applicable	1046	41	3.9
6. Reproductive status			
a. Pregnant	556	32	5.8
b. Not pregnant	1270	70	5.6
c. Castrated	337	21	6.2
d. Not castrated	583	15	2.6
<2m-postpartum	50	4	8.0
<6m-age(calves)	74	3	4.1

Table 5.6 (b): Univariate analysis of animal level variables for c-ELISA positive domestic ruminants.

	Parameter Estimates				*95% C.I.*		
Variable	*Coefficient*	*Std.Error*	*p-value*	*Odds Ratio*	*Lower*	*Upper*	*LRS*
Domestic ruminants							
a. Cattle	Ref			1.00			
b. Goats	0.43	0.19	0.02	1.54	1.07	2.23	
c. Sheep	0.19	0.25	0.45	1.21	0.74	1.98	0.07
Sex							
a. Male	Ref			1.00			
b. Female	0.26	0.19	0.18	1.30	0.89	1.99	0.17
Age by dentition							
a. 0				1.00			
b. 1	0.56	0.35	0.11	1.76	0.88	3.52	
c. 2	0.83	0.34	0.02	2.30	1.18	4.48	
d. 3	1.15	0.35	< 0.001	3.16	1.60	6.23	
e. 4	1.03	0.25	< 0.001	2.80	1.73	4.51	<0.001
Domestic ruminant source							
a. Homebred	Ref			1.00			
b. Brought in	0.19	0.30	0.52	1.2	0.67	2.20	0.53
Domestic ruminant abortion							
a. Yes	Ref			1.00			
b. No	-0.49	0.25	0.05	0.61	0.38	0.99	<0.001
Reproductive status							
a. Pregnant	Ref			1.00			
b. Not pregnant	-0.29	0.24	0.23	0.75	0.46	1.20	
c. Castrated	-0.30	0.32	0.36	0.74	0.40	1.39	
d. Not castrated	-0.68	0.34	0.04	0.50	0.26	0.98	

e. <2mpp	0.34	0.60	0.58	1.40	0.43	4.54	
f.<6m-calves	0.06	0.67	0.93	1.06	0.29	3.91	0.35

Potential household level variables observed and reported by household members are shown in Table 5.7a. The herd or flock level variables found to be associated with increased risk of c-ELISA seropositivity during univariate analysis were parturition in the boma and in grazing areas, herd to herd contact, animal-wildlife contacts, acquiring animals, herding all animals together, presence of wildlife animals in grazing areas, farm types, herd size and feeding aborted foeti and placenta to dogs (Table 5.7b).

Table 5.7(a): Distribution of c-ELISA positives by household level variables.

Variable	*Domestic ruminants tested*	*Domestic ruminants c-ELISA positive*	*%positives*
1. Herd size			
a.1-40	1627	39	2.4
b. 41-80	512	21	4.1
c. 81-120	561	69	12.3
d. 121-160	229	4	1.7
e. 161-200	221	32	14.5
d. >200	221	13	5.9
2. Farm types			
a. Pastoral	1948	161	8.3
b. Agro-pastoral	1439	17	1.2
3. Acquire domestic ruminants in 2001			
a. Yes	1751	116	6.6
b. No	1636	62	3.8
4. Using sun-dried manure as bedding			
a. Yes	1618	38	2.3

b. No	1769	140	7.9
5. Foetus and placenta fed to dogs			
a. Yes	959	110	11.5
b. No	2428	68	2.8
6. Throw away placenta			
a. Yes	250	20	8.0
b. No	3137	158	5.0
7. Parturate inside			
a. Yes	897	11	1.2
b. No	2490	167	6.7
8. Parturate in grazing areas			
a. Yes	2037	144	7.1
b. No	1178	30	2.5
c. Not applicable	172	4	2.3
9. Parturate in the boma			
Yes	2126	150	7.1
No	1104	24	2.2
NA	157	4	2.5
10. Parturate in dry season			
a. Yes	2135	118	5.5
b. No	1252	60	4.8
11. Partuate in wet season			
a. Yes	2641	149	5.6
b. No	746	29	3.9
12. Livestock-wildlife contact dry season in grazing areas			
a. Yes	2282	167	7.3
b. No	1105	11	0.1
13. Livestock-wildlife contact wet season in grazing areas			
a. Yes	2324	169	7.3
b. No	1063	9	0.8
14. Herd-Herd contact dry season in grazing areas			
a. Yes	3180	177	5.6
b. No	207	1	0.5

15. Herd-Herd contact wet season in grazing areas			
a. Yes	3126	177	5.7
b. No	261	1	0.4
16. Herding all dry season			
a. Yes	1482	37	2.5
b. No	1619	130	8.0
c. Not applicable	286	11	3.8
17. Herding all wet season			
a. Yes	1454	36	2.5
b. No	1751	135	7.7
c. Not applicable	182	7	3.8
18. Buffalo in grazing areas			
a. Yes	1303	113	8.7
b. No	2084	65	3.1
19. Dikdik in grazing areas			
a. Yes	3037	171	5.6
b. No	350	7	2
20. Wildebeest in grazing			
a. Yes	1347	138	10.2
b. No	2040	40	2.0
21. Zebra in grazing areas			
a. Yes	1526	143	9.4
b. No	1826	35	1.9
22. Impala in grazing areas			
a. Yes	1943	152	7.8
b. No	1444	26	1.8
23. Giraffe in grazing areas			
a. Yes	1750	151	8.6
b. No	1637	27	1.6
24. Thomson-gazelle in grazing areas			
a. Yes	1740	149	8.6
b. No	1647	29	1.8

Table 5.7b: Univariate analysis of household level variables for c-ELISA positive domestic ruminants.

	Parameter Estimates				*95% C.I.*		
Variable	*Coefficient*	*Std.Error*	*p-value*	*Odds Ratio*	*Lower*	*Upper*	*LRS*
Herd size							
a. 1-40	Ref			1.00			
b. 41-80	0.77	0.44	0.08	2.16	0.92	5.07	
c. 81-120	1.37	0.36	< 0.001	3.92	1.96	7.86	
d. 121-160	-0.10	0.87	0.91	0.90	0.17	4.92	
e. 161-200	1.29	0.37	< 0.001	3.65	1.8	7.54	
f. >200	1.06	0.41	0.01	2.87	1.28	6.44	< 0.001
Farm types							
Pastoral	Ref			1.00			
Agro-pastoral	-2.02	0.33	< 0.001	0.13	0.07	0.26	< 0.001
Acquire domestic ruminants							
a. No	Ref			1.00			
b. Yes	0.91	0.22	< 0.001	2.49	1.62	3.82	<0.001
Using sun-dried manure as bedding							
a. No	Ref			1.00			
b. Yes	-1.09	0.25	< 0.001	0.34	0.21	0.54	<0.001
Foetuses & placenta fed to dogs							
a. No	Ref			1.00			
b. YES	1.02	0.22	< 0.001	2.79	1.8	4.31	< 0.001
Throw away placenta							
a. No	Ref			1.00			
b. Yes	1.80	0.44	< 0.001	6.05	2.57	14.24	0.1811
Parturate inside							
a. No	Ref			1.00			
b. Yes	-1.69	0.45	< 0.001	0.18	0.08	0.44	< 0.001
Parturate in grazing areas							
a. No	Ref			1.00			

b. Yes	1.08	0.29	< 0.001	2.96	1.68	5.23	< 0.001
Parturate in boma							
a. No	Ref			1.00			
b. Yes	1.16	0.28	<0.001	3.20	1.84	5.57	<0.001
Parturate in dry season							
a. No	Ref			1.00			
b. Yes	0.16	0.31	0.61	1.17	0.64	2.15	0.5968
Parturate in wet season							
a. No	Ref			1.00			
b. Yes	0.70	0.27	0.009	2.02	1.19	3.41	0.0485
Livestock-wildlife contact in dry in grazing areas							
a. Yes	Ref			1.00			
b. No	-2.03	0.40	< 0.001	0.13	0.06	0.29	< 0.001
Livestock-wildlife contact in wet in grazing areas							
a. Yes	Ref			1.00			
b. No	-2.03	0.40	< 0.001	0.13	0.06	0.29	< 0.001
Herd-herd contact in dry in grazing areas							
a. Yes	Ref			1.00			
b. No	-2.71	1.33	0.04	0.07	0.01	0.91	0.0173
Herd-herd contact in wet in grazing areas							
a. Yes	Ref			1.00			
b. No	-2.72	1.14	0.02	0.066	0.01	0.62	0.0025
Herding all in dry season							
a. No	Ref			1.00			
b. Yes	-0.91	0.28	0.001	0.40	0.23	0.70	< 0.001
c.Not applicable	-0.92	0.37	0.013	0.40	0.19	0.85	
Herding all in wet season							
a. No	Ref			1.00			
b. Yes	-0.98	0.29	< 0.001	0.38	0.21	0.67	<0.001
c. Not applicable	-0.93	0.37	0.012	0.40	0.19	0.82	
Buffalo in grazing areas							
a. Yes	Ref			1.00			

b. No	-1.29	0.22	< 0.001	0.28	0.18	0.42	<0.001
Dikdik in grazing areas							
a. Yes	Ref			1.00			
b. No	-1.10	0.50	0.029	0.33	0.12	0.89	0.0332
Wildebeest in grazing areas							
a. Yes	Ref			1.00			
b. No	-1.39	0.29	< 0.001	0.25	0.14	0.44	<0.001
Zebra in grazing areas							
a. Yes	Ref			1.00			
b. No	-1.45	0.29	< 0.001	0.24	0.13	0.42	<0.001
Impala in grazing areas							
a. Yes	Ref			1.00			
b. No	-1.36	0.29	< 0.001	0.26	0.15	0.46	<0.001
Giraffe in grazing areas							
a. Yes	Ref			1.00			
b. No	-1.50	0.29	< 0.001	0.22	0.13	0.39	<0.001
Thomson in grazing areas							
a. Yes	Ref			1.00			
b. No	-1.42	0.28	< 0.001	0.24	0.14	0.42	<0.001
Altitude	0.00	0.00	0.18	1.00	1.00	1.00	0.3334
Neighbour distance	0.00	0.00	0.99	1.00	1.00	1.00	0.9847

The final model for c-ELISA positive households included feeding dogs with aborted foeti and placenta (OR = 2.3, 95%CI = 1.22, 4.29; p<0.001) type of farming system (OR = 0.11, 95%CI = 0.048, 0.263, p<0.001) and parturition during wet season (OR = 0.29, 95%CI = 0.122, 0.693, p= 0.0065) (Table 5.8). The model produces a deviance of 1175.5 with 1 degree of freedom (df) and likelihood ratio statistic of 0.0065.

Table 5.8: A final model for c-ELISA positive domestic ruminants during 2002 and 2003 in Arusha and Manyara regions

Variable	*B*	*SE*	*LRT P-value*	*OR*	*95% CI*
Aborted foeti and placenta fed to dogs	0.83	0.322	<0.001	2.3	1.216, 4.293
Farm type-(agro-pastoral)	-2.19	0.436	<0.001	0.11	0.048, 0.263
Parturition during the wet season	-1.23	0.443	0.0065	0.29	0.122, 0.693

The results (Table 5.8) show that feeding dogs with placenta and aborted foetus was independently and significantly associated with a greatly increased odds of infection (adjusted OR = 2.3). Parturition during the wet season and agro-pastoral farming system were negatively associated with c-ELISA positivity at household level.

5.3.2 Human c-ELISA seropositivity

During the cross-sectional survey, 104 families were visited. Fourteen families were not bled due to non-compliance or because family members were far from the animal enclosures. Therefore, 90 families with a total of 476 family members were screened. Within these families however, young children who were afraid and those individuals failing to comply were not bled. Seventy four percent of the families had family members ranging from 1-6 who complied for bleeding. The distribution of family members sampled per family is shown in Figure 5.7a.

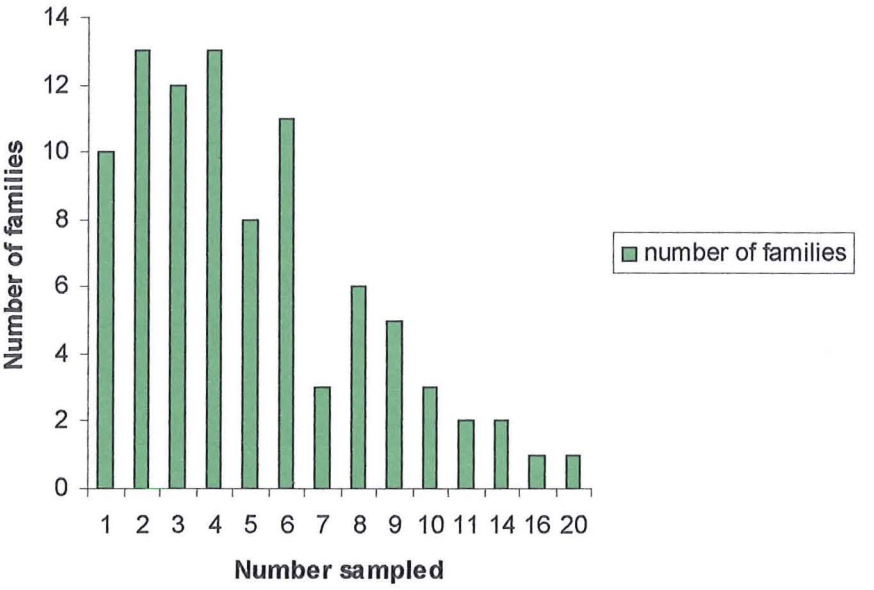


Figure 5.7a: Frequency distribution of family members sampled per family

Of those screened, 460 sera were tested using c-ELISA with 38 (8.3%) positive. There was no statistical difference seropositivity in males and females (χ^2 =0.19, p = 0.663). A higher proportion of human c-ELISA seropositivity was observed in the agro-pastoral farming system (8.7%) than in the pastoral system (7.4%) though the difference was not statistically significant (χ^2 =0.23, p = 0.631). The highest proportion of human c-ELISA positive families was observed in Ngorongoro district (46%) and lowest in Babati district (0%). All family members (n = 56) from 12 families sampled in Babati district were c-ELISA negative (Table 5.9). The spatial distribution of families' serostatus is shown in Figure 5.7b.

Table 5.9: C-ELISA seropositivity in humans by district, family and individual level

District	*Families screened*	*Families positive*	*%positive families*	*People screened*	*People positive*	*%people positive*
Babati	12	0	0	56	0	0
Hanang	13	3	23.08	49	3	6.12
Mbulu	18	2	11.11	92	3	3.26
Karatu	34	14	41.18	180	24	13.33
Ngorongoro	13	6	46.15	83	8	9.64
Total	90	25	27.78	460	38	8.26

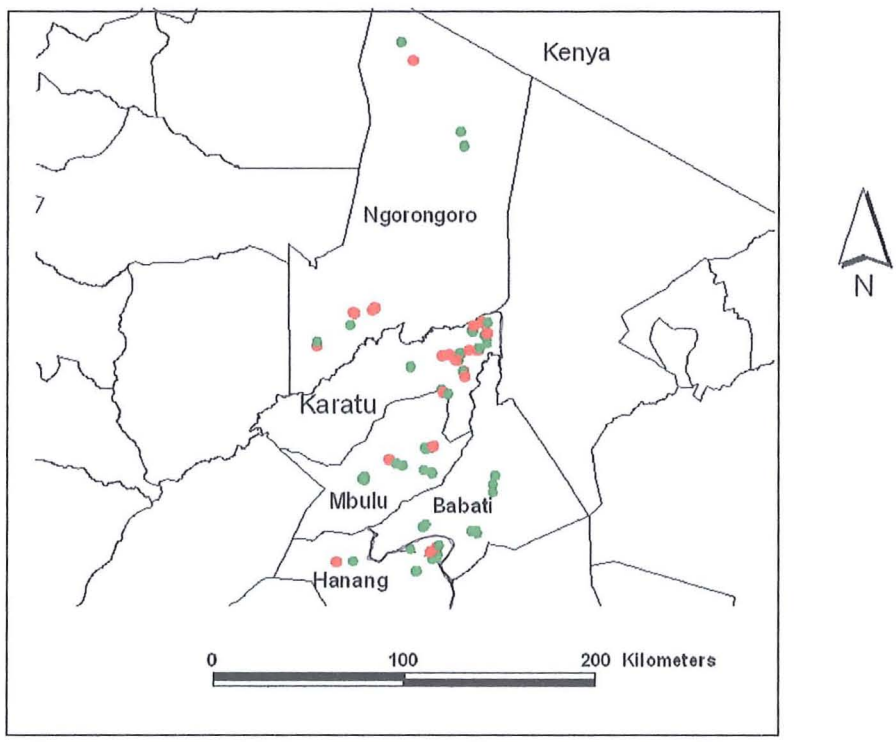


Figure 5.7(b): Spatial distribution of families with c-ELISA serostatus in 2003

Circles: Red = Positive families Green = Negative families

5.3.2.1 Relationship between livestock infection and human infection

Fifty two percent (13/25) of families that were c-ELISA positive also had infected herds and flocks. In addition 25% (16/65) of families that were c-ELISA negative had infected herds and flocks. There was a significant association between c-ELISA seropositivity in families and c-ELISA seropositivity in households (OR = 3.3, 95%CI = 1.26, 8.67, $p<0.05$) (Figure 5.8). Family members in the c-ELISA positive households were 3.3 (OR) times more likely to be c-ELISA positive than those in seronegative livestock households. It has been found that 48% (12/25) of families were c-ELISA seropositive while their herds and flocks were c-ELISA seronegative.

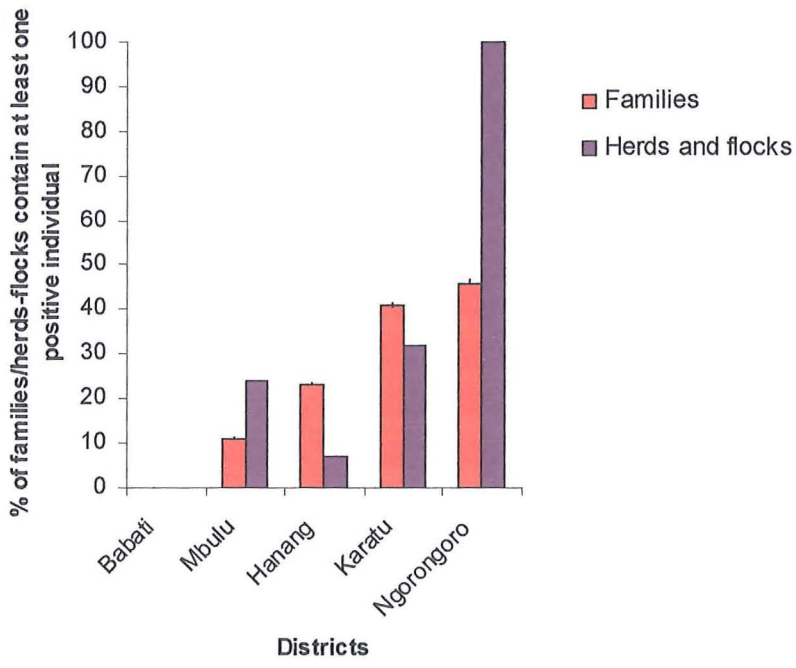


Figure 5.8: C-ELISA seropositivity in families and livestock-keeping households.

5.4 Discussion

The results of the current study indicated that *Brucella* infection is widespread in both livestock and human populations of the Manyara and Arusha regions of Northern Tanzania. The current proportion of c-ELISA seropositive cattle was lower (4.7%) than shown by Staak and Protz (6.9%) (1973) from the same area probably due to different sampling techniques and to the screening test used. Recent studies conducted by Niwael (2001) and Minja (2002) in Babati and Hanang districts revealed a seroprevalence of 3% in cattle. The fact that the seroprevalence observed by Minja (2002) and Niwael (2001) differed from the current results may be due to the relatively small sample sizes and that the study was concentrated on agro-pastoral cattle in only two districts. The current findings are likely to be a better representation of the infection in domestic ruminants in Manyara and Arusha regions in both the pastoral and agro-pastoral farming systems because of the increased area coverage and the inclusion of small ruminants.

Studies conducted in some of the African countries including Tanzania, showed variable seroprevalence such as 6.5% in Sudan (Hellmann *et al.*, 1984), 6.6% in both Nigeria and Ghana (Ocholi *et al.*, 1996; Kubuafor, *et al.*, 2000) , 10% in Kenya (Kadohira, *et al.*, 1997) and 12% in Tanzania (Weinhaupl, *et al.*, 2000). The different seroprevalences observed in different regions of Tanzania and in other countries may be due to the level of risk factors that may vary from one geographical location to another within similar farming systems. Different levels of herd to herd interaction, climatic conditions which favour survival of *Brucella* organisms for long periods and the time the infection was introduced into the herd or flock prior to testing may result in different seroprevalences within farming systems in

various places. In addition, different sampling methods and techniques and the diagnostic tests employed may result in different seroprevalences within similar farming systems.

A high proportion of c-ELISA seropositivity in goats observed in the current study was not consistent with other studies conducted in Tanzania where cattle had a higher seroprevalence than small ruminants (Fison, 1986 unpublished; Mahlau and Hammond, 1962; Mahlau, 1967; Niwael, 2001). This may be due to the fact that most of the studies focused on screening cattle rather than small ruminants. A higher proportion of c-ELISA seropositivity in goats than in sheep is similar to other studies where *Brucella* seroprevalence in sheep was low (Mahlau, 1967; Dessai, *et al.*, 1995; Omer, *et al.*, 2000b). The low seroprevalence in sheep may be attributed to lower susceptibility to brucellosis shown by certain breeds of sheep (Bishop, *et al.*, 1994; Acha and Szyfres, 2001). Eating behaviour may also be a protective factor for sheep, as they often graze in a more restricted manner than goats whose active foraging for food may increase the risk of exposure to brucellosis. The role of small ruminants in maintaining and transmitting brucellosis among domestic ruminants should not be underestimated especially in pastoral animals where the goats seropositivity was high. In pastoral settings, cattle and small ruminants share similar watering points, grazing grounds and holding grounds (boma). This close contact could have a substantial effect on transmission of brucellosis from small ruminants to cattle and *vice-versa*. Under such circumstances, the presence of *B. melitensis* in small ruminants and *B. abortus* in cattle could cross-infect cattle and small ruminants which, in turn, infect humans resulting in a severe human brucellosis. Presence of *B. melitensis* type 1 in goats reared in pastoral farming systems in this study, raises the potential for transmission to cattle. An indication that cross-species transmission may occur was provided by the fact

that herding domestic ruminants together was associated with c-ELISA seropositivity. Although small ruminants have been ignored in many previous studies (Staak and Protz, 1973; Weinhaupl *et al.*, 2000), the current results emphasize the importance of including small ruminants during brucellosis screening in pastoral systems.

The age-related increase in seroprevalence is consistent with an endemic pattern of infection in animals. In an endemic situation (where infection is continuously present), animals are increasingly likely to have become exposed with time and hence show increasing positivity with age. Another explanation could be the ability of some young animals to clear the infection or the fact that infections may remain latent until adulthood when the animal is sexually mature (Bishop, *et al.*, 1994; Radostits *et al.*, 2000). It has been suggested that susceptibility to brucellosis is commonly associated with sexual maturity rather than age, as certain breeds that mature earlier in life become susceptible to brucellosis at an earlier age than those that mature later (Crawford. *et al.*,1990; Bishop, *et al.*, 1994; Radostits, *et al.*, 2000). Therefore, cattle such as Tanzanian short horn Zebu (*Bos indicus*) that mature late may be less susceptible to infection in the herd than exotic cattle (*Bos taurus*).

As in other studies in many parts of the world, higher seroprevalences were recorded in larger herds and flocks (Hellmann *et al.*, 1984; Kadohira, *et al.*, 1997). This might be expected if densities and contact rate (and hence the potential of transmission) increase with increasing numbers of individuals. Lack of associations between c-ELISA seropositivity and flock size in the agropastoral farming system could be due to several

flocks having small number of animals. For examples 64% of agro-pastoral flocks screened consisted of sheep and goats ranging from 1-20 in number.

The effect of brucellosis on reproductive performance was studied by calculating the parturition intervals and abortions in cattle and small ruminants. Although parturition intervals were high with a mean of 16.3 months in cattle and 12.5 months in small ruminants, there was no statistical association between long parturition interval and c-ELISA seropositivity ($p = 0.809$). The calving interval of 16.3 months in Zebu is longer than the recommended calving interval of 12-13.5 months for Zebu cattle and 12-13 months in Holstein cattle (Esslemont, 1992; Rege, *et al.*, 2001). Ewes and does give birth twice per year but this occurred once per year in the current study. Poor nutrition especially during the dry season, lack of water and various stress factors may affect both fertility and production. Similar observations were reported by Whitaker, *et al.*, (1993) where poor nutrition contributed to poor fertility in Irish dairy cattle.

Long parturition intervals in small ruminants may result from interventions by flock owners who control the breeding cycle using skin or plastic sheaths (Figure 5.9). Breeding control by flock owners was to ensure parturitions coincided with the rainy season. Although there was no association between parturition intervals and c-ELISA seropositivity in this study, other studies have demonstrated an association in dairy cattle in Tanzania (Swai, 1997).

Figure 5.9: The use of skin and plastic sheaths to control breeding in pastoral small ruminants

C-ELISA seropositivity in cattle was associated with a history of abortion and was in agreement with several other studies (Swai, 1997; Kubuafor *et al*,. 2000; Schelling *et al*., 2003). The attributable risk of 12% observed in this study, indicates that brucellosis causes abortions especially in herds with high levels of infection (pastoral households were 15 times more likely to have abortion compared to agropastoral households) although a range of other disease conditions including tick borne diseases and typanosomosis may also cause abortion in cattle (Radostits, *et al*., 2000). The association between a history of abortion, retained placentae and c-ELISA seropositivity in pastoral animals was consistent

with other studies Schelling, *et al*., 2003). Abortion and retain placentae resulted in high loss of young stock for replacement and monetary expenditure in treating retained placentae (Chapter VII) and occasionally metritis. No individual animal had a history of retained placenta or abortion and was c-ELISA seropositive among agropastoral animals. These findings suggest that brucellosis was not likely to be the principal cause of abortions and retained placenta that occurred in the households, or alternatively, information provided by owners may not be accurate due to them not keeping livestock records.

The spatial distribution of herds and flocks with c-ELISA seropositivity varied between districts in the study area. Of the five districts surveyed, c-ELISA seropositivity ranged from 0-9% at the individual animal level and 0-30% at the household level. Ngorongoro district had a significantly higher level of c-ELISA seropositive animals and households than other districts ($P<0.001$). The difference was due to the farming systems as Ngorongoro district is a pastoralist district, while Karatu, Mbulu and Hanang districts are principally agro-pastoral, although a few villages practice pastoralism. All villages visited in Babati district were practicing agro-pastoralism and their animals were c-ELISA seronegative. Lack of seropositive animals in Babati district could be explained by the management system where there were few animals per household (average of 22 animals/household) compared to other districts (ranged from 33-120 animals/household) was kept in small areas of land where interaction between animals from different households was limited. This was comparable to results obtained by Minja (2002) who found a lower brucellosis seroprevalence in Babati compared to Hanang district and Kadohira *et al*., (1997) who observed low seroprevalence in Kenya in districts where the crop-livestock farming systems predominated.

The high variation in c-ELISA seropositivity between households within the pastoral farming system may be due to variations in herd and flock characteristics, such as the number of mature infected females present, herd or flock size, stocking density and general hygiene. A greater homogeneity in agro-pastoral households with a high proportion of uninfected households, suggests that some of the risk factors responsible for brucellosis occurrence and transmission may not be present in this system. For example small herd and flock sizes, semi-intensive rearing, infrequent introduction of animals and low herd to herd interactions could be protective factors against the occurrence of brucellosis in agro-pastoral farming systems.

Several managemental risk factors were significant in the univariate analysis but not in the multivariate model but it is worth discussing them to explore their implications within farming systems. In the current study it was found that herds and flocks giving birth in grazing areas were 3 times more likely to be c-ELISA positive compared to those giving birth inside and in the boma. Similarly, those giving birth in the boma were 3 times more likely to be c-ELISA positive compared to those that gave birth inside and in the grazing areas. Parturition in the grazing areas may contaminate the environment, providing a source of infection for other animals in the area. Congregation of animals in the boma enhances cross-licking due to close proximity following parturition, and thus animals may be contaminated by brucellosis. The problem may be due to the fact that survival of *Brucella* organisms may be prolonged, thus animals may become exposed to the infective agent for longer periods of time. Similar suggestions were put forward by Bishop, *et al.*,

(1994) where humidity and temperatures appeared to influence the organism's ability to survive in the environment.

It was also observed from this study that animals brought into the herd or flock were associated with increased risk of c-ELISA seropositivity. This may be explained by the fact that new animals are not screened prior to introduction, thus infected animals may spread the disease in the herds or flocks following parturition. Similar findings were reported where introduction of replacement animals into the herd and flock was positively associated with c-ELISA seropositive herds and flocks (Bishop, *et al.*, 1994; Lithg-Pereira *et al.*, 2004).

Contacts between herds and flocks from different households were observed to be associated with an increased risk of c-ELISA seropositivity in the study area. Herd to herd contact is not uncommon in pastoral areas especially during the dry season. Congregation in communal areas occurred when pastures and water became scarce during the dry period, animals were transferred to new and limited areas with some pastures and water and this resulted in many animals sharing the same pastures and water points. In the dry period, relatively high stocking rates are not uncommon in the few available places where pastures and water can sustain animals through the dry period. Such congregation allows for increased contamination of pastures and easy cross-infection within and between herds and flocks once abortion and delivery occurs.

The results observed that contact between domestic and wildlife ruminants in grazing areas were statistically associated with c-ELISA seropositivity, especially when herds and flocks

came in contact with buffalo, wildebeest, zebra, impala, Thomson gazelle and giraffe. It was observed that pastoral animals kept in Ngorongoro district are usually grazed with wild ruminants such as impala, Thomson gazelle, giraffe and zebra (Personal observation 2003). Although herd owners do not graze livestock close to wildebeest to avoid Malignant Catarrhal Fever, sporadic pasture contamination can not be avoided. Therefore, transmission of brucellosis between the two populations may be possible due to such close associations. Comparable studies have shown transmission of infection from wildlife animals to cattle had occurred in USA following pasture contamination with wild elk (Hillman, (2002). Hillman, (2002) isolated *Brucella abortus* biovar 1 from both wild elk tissues and cattle milk. Further evidence was observed in Wood Buffalo National Park, Canada where *Brucella abortus* biovar 1 was isolated from both cattle and bison (Forbes and Tessaro, 1996). A notable problem of brucellosis transmission from bison to cattle was reported in the Yellowstone National park, USA where it was a threat to livestock keepers as brucellosis had been eradicated in livestock in the area (Thorne, 2004). Therefore in other places where livestock and wildlife share similar resources or come into contact transmission between domestic and wild animals is expected (Waghela and Karstad, 1986; Madsen and Anderson, 1995).

Interesting results from the current study were the statistical association observed between feeding dogs with placenta and aborted foeti and c-ELISA seropositivity with the risk in these households being 3 times more likely to be c-ELISA positive compared to households who did not feed placenta and aborted foeti to dogs. The c-ELISA seropositivity was associated with dogs eating reproductive materials probably because dogs tend to carry pieces of placenta and aborted foeti from one place to another and thus

contaminate the environment, especially the surrounding pastures. Based on the nature of pastoral households, dogs carry such materials outside the boma and contaminate grasses nearby which in turn, animals graze while turned out onto the grazing areas. The role of dogs in the epidemiology of bovine brucellosis was suggested by Forbes (1990) and an association demonstrated between infected dogs and outbreak of brucellosis in cattle. In the current study however, the spread of organisms in the environment by dogs was probably more important than infected dogs themselves.

Other interesting findings were that parturition inside (OR = 0.18), agro-pastoral farming system (OR = 0.13) and practice of taking manure out to dry and then taking it back as bedding (OR = 0.34) was associated with a reduced risk of brucellosis at the household level. Parturition inside was negatively associated with c-ELISA seropositivity based on the fact that family members have the habit of cleaning their houses (cohabited houses) every day thus removing all materials collectively and accumulating them outside. Taking manure outside and letting it dry before taking it back as bedding could affect the survival of *Brucella* organisms and thus reduce the infection levels. The agro-pastoral farming system was a protective factor based on the nature of management as described previously.

During multivariate analysis, feeding dogs with placenta and aborted foeti was positively associated with c-ELISA seropositivity with adjusted odds being slightly less than the univariate odds. Presence of this variable in the final model emphasizes its role in the transmission of brucellosis. However, to have aborted foetuses alone was associated with c-ELISA seropositivity, thus more likely to occur in seropositve households. Agro-pastoral farming system and parturition during the wet season were associated with the reduced risk

of brucellosis. The explanations for agro-pastoral farming systems being negatively associated with c-ELISA seropositivity were as described above. Parturition during the wet season was negatively associated with c-ELISA seropositivity when adjusted for the effects of feeding dogs with placenta and foetuses and farming systems during multivariate analysis although it was positively correlated with c-ELISA seropositivity during univariate analysis. Therefore, farming systems, parturition during the wet season and feeding dogs with placentae and aborted foeti were the main three risk factors that explained the occurrence of brucellosis in the study area.

The overall c-ELISA seropositivity in humans was 8.3%. This is the first and highest figure to be reported in a cross-sectional survey in pastoral and agro-pastoral communities in Tanzania. The cross-sectional study by Minja (2002) found that livestock keepers were infected (0.7%) among different groups of people who handle livestock and livestock products in Babati and Hanang districts. Similarly this study was conducted mainly in the agro-pastoral areas. Therefore, the current study encompasses both pastoral and agro-pastoral families and had a wider coverage thus resulting to a higher seroprevalence than the previous studies. Several studies carried in other countries indicated variable seroprevalences based on the rate of infection in animals such as 18-24% in humans and 18% in farms in Uganda (Ndyabahinduka and Chu, 1984), 3.8% in humans and 7% in cattle in Chad (Schelling *et al.*, 2003) and 40 cases/100,000 in humans and 15% in animals in Saudi Arabia (Memish, 2001). The variations of seroprevalence between humans and livestock could be probably due to the extent of spread of the disease in livestock populations and risk factors associated with transmission of brucellosis from animals to humans.

Absence of c-ELISA seropositive families in Babati observed in this study was consistent with the previous studies where the seroprevalence was low compared to Hanang district (Niwael, 2001). This could be explained by the fact that domestic ruminants were also c-ELISA seronegative during cross-sectional screening, and other studies have also indicated low seroprevalence in Babati district (Minja, 2002). This was supported by the fact that human brucellosis occurred when brucellosis was present in livestock populations. Families with the highest c-ELISA seropositivity was observed in Ngorongoro district which is a pastoral district, followed by other districts which are predominantly agro-pastoralist. This was expected because in all families that were screened in Ngorongoro district their herds and flocks were also c-ELISA positive. Close cohabitation under poor hygiene, eating habits and livestock related activities performed without protective measures could have resulted in high family seroprevalence in the district. Assisting with parturition and handling aborted foeti and retained placenta may be risk factors for human infection as these were found to be significant in the livestock c-ELISA seropositivity final model. This was further supported by the fact that there was a significant statistical association between families with c-ELISA seropositivity and herd c-ELISA seropositivity. Furthermore, 48% percent of families were c-ELISA seropositive while their herds and flocks were c-ELISA seronegative. Family members could acquire infection from neighbours through drinking raw milk, assisting parturitions or handling aborted materials and in livestock auction markets where people may have access to raw blood, milk and meat. It was also observed that 25% of families were c-ELISA seronegative yet their livestock were seropositive. One explanation could be the fact that in some families not all members were tested resulting in false negative families. This may

mask the real status of the disease at family level. These families were from agro-pastoral farming systems where some households kept high numbers of male rather than female animals for transport and draught purposes. Therefore, risk from infected males is probably minimal as humans acquire infection through consumption of raw milk and handling foetal materials and placentae. Also the practice of boiling milk may be common in these households thus reducing the risk of human infection. Another possible explanation could be the recent introduction of infected animals into the herd or flock.

CHAPTER VI

6.0 BRUCELLOSIS INFECTION DYNAMICS AND IMPACT ON PRODUCTION AND REPRODUCTION IN DOMESTIC RUMINANTS

Abstract

A longitudinal study was conducted in pastoral herds and flocks and one beef ranch in order to elucidate the dynamics of brucellosis and its impact on abortion, retained placenta and milk production for a period of twelve months. Initially, 469 animals were enrolled for the study. Animals were bled every three months to determine the incidence rate of brucellosis c-ELISA seropositivity, to evaluate trends in serostatus, and to estimate survival probability. In addition individual animal reproductive information was collected. Milk yield was measured indirectly by estimating the growth rate of calves by taking the heart girth measurements every three months.

Forty seven new c-ELISA seropositive animals were identified over the period of three months representing an estimated incidence of 0.732 (732/1,000) cases per animal-years at risk. The estimated survival probability over twelve months was 0.836. Households with a high seroprevalence at the initial sampling were observed to have high infection rate in the subsequent visit. A statistical association between the occurrence of new c-ELISA seropositive cases and season was observed (P = 0.018), with a high incidence reported during the wet season. Furthermore positive to negative seroconversion was observed in 16 female animals.

Of the 94 female animals that were expected to parturate, 15% aborted with 29% of aborting animals being c-ELISA seropositive. Retained placenta was observed in 4.3% of the domestic ruminants. The cost of treating retained placenta ranged from Tshs. 1000-4000 (US$ 1-4) with an average of Tshs. 2400 (US$ 2.4).

Of the 79 calves that were screened, 21.5% were c-ELISA seropositive. Eighty two percent of the c-ELISA seropositive calves were born from seropositive dams. Calves born from seropositive dams were 27 (OR = 27) times more likely to be seropositive than those from seronegative dams.

There was no statistical significant difference (P>0.05) in growth rate between calves suckling from c-ELISA seropositive and seronegative dams.

6.1 Introduction

In animals, brucellosis has the potential to cause enormous economic loss through abortion, decreased milk yield, placental retention and impaired fertility (Antoniou, *et al.*, 2002). Previous studies evaluating the impact of disease have been confined to dairy herds Mdoe *et al.*, 1991) and there are limited studies that have been extended to extensive farming systems (Mokantla, *et al.*, 2004). It has been observed that in the pastoral farming system, the real inputs and economic outputs are often not well known by herd owners (Mokantla, *et al.*, 2004). This, together with lack of record keeping and significant livestock movements complicates any evaluation strategy on production and reproduction status at the herd level. Evaluation based on financial loss caused by brucellosis becomes

even more difficult due to the differing nature of farming systems, varying herd sizes (Antoniou, *et al.*, 2002) and the purpose of livestock keeping such as prestige, social and cultural functions. Also in extensive farming systems, the causes of abortion and retained placenta are numerous, (Arthur, *et al.*, 1989; Mokantla, *et al.*, 2004) where other infectious diseases and management factors have been shown to play a major role (Swai, 1997).

Livestock have a direct impact on the health and social well-being of pastoralists whose livelihoods are dependent upon livestock and livestock products. Low milk production may result in malnutrition especially in children who depend heavily on consumption of milk. High abortion rates result in small numbers of replacement stock which lead to decreased herd sizes and thus to poverty. Extra costs incurred for treating retained placenta and sometimes metritis increases the economic burden to livestock keepers. The effect of brucellosis on milk yield has been quantified and found to significantly reduce yield to below average in dairy animals in Ethiopia (Sintaro, 1994). No similar study has been conducted in Tanzania in any farming system to quantify the impact of brucellosis on milk yield, retained placenta and abortion. Thus, quantifying abortion rates, milk production and the incidence of retained placenta attributed to brucellosis could generate useful information for future formulation of appropriate control measures that ultimately may alleviate poverty in the sector.

Therefore, this study aims to:

(i) Study the dynamics of brucellosis in domestic ruminants.

(ii) Determine the impact of brucellosis on abortion and retain placenta in domestic ruminants and milk yield in cattle.

6.2 Materials and methods

The study was conducted in pastoral households of Arusha region for 12 months, from September, 2002 to December, 2003. Six households were selected for longitudinal study base on convenience. The following criteria were used to select the longitudinal households:

(i) Households with ≥100 animals and FRBPT seropositivity ≥10%.

(ii) Compliance with herd owners.

Households with a high seroprevalence were chosen so to give the greatest chance of detecting the impact of brucellosis within these households. Four households were selected from the cross-sectional study (Chapter V). To increase the number of households in the longitudinal study, one beef ranch and one household that were not included in the cross-sectional study were also enrolled.

Animals were bled every three months for a duration of twelve months. Information at the individual animal and herd level was obtained from the owner. Individual animal information included abortion, retained placenta following normal delivery or abortion, deliveries, cost of treating retained placenta and pregnancy status were collected every three months. Replacement of animals which dropped out of the study was performed by restraining any animal from the herd or flock. Newly recruited animals were tagged for identification. However, in some households the owner was reluctant to tag the new recruits. In such households replacement by recruiting other animals in the household was limited. Some individual owners would not allow animals to be bled during the dry season.

Calf growth rate was estimated by measuring their heart girth using a measuring tape. Calves stood squarely on four legs while restrained; the measuring tape was placed around the animal just behind the hump and forelegs, and heart girth measurements taken. Heart girth measurements were carried out at three months intervals. An increase in girth measurement (cm) was considered as an increase in growth. Seventy nine calves were enrolled in the study. Each calf was matched with the respective dam serostatus.

6.2.1 Data storage and analysis

Data were entered in the Microsoft Excel 97 spread sheet. One way analysis of variance (ANOVA) was used to determine any difference between seasonal incidences and descriptive analysis used to calculate percentage proportions for sex, parturition, retained placentae and abortion. Figures were produced with Microsoft Excel. The increase in heart girth measurements for calves suckling from *Brucella* seronegative and seropositive dams were compared for any difference using the Wilcoxon test. The incidences and survival probabilities were calculated as described by Thrusfield (1995) and Woodward (2005).

$$\text{Incidence Rate (IR)} = \frac{\text{Number of new cases in the three months period}}{[(\text{Number of domestic ruminants at risk at start of the time period} + \text{Number of domestic ruminants at risk at the end of that period})/2)]}$$

'A new case' in this study refers to any animal that seroconverted from being c-ELISA seronegative to c-ELISA seropositive.

The incidence rate estimated at the first three months (period x) was extrapolated to a period of twelve months (period y). Therefore, $\mathbf{I_y = I_x(y/x)}$ where $\mathbf{I_y}$ = Incidence for one

year and I_x = incidence for the first three months. The relationship between baseline seroprevalence and incidence rate was assessed by using Pearson correlation coefficient.

6.3 Results

6.3.1 Infection dynamics

At the beginning of the study, six livestock households were enrolled with 332 negative and 137 seropositive domestic ruminants respectively. During the period of twelve months loss to the study of animals occurred in both seronegative and seropositive domestic ruminants. Loss to the study of animals occurred due to several factors ranked in terms of number of animals lost and included movements to sites where visiting and sampling was not possible, sale or gifting, slaughter, deaths, or attack by wild animals. The maximum number of domestic ruminants lost to the study between visits was 33%. This occurred as one herd sold more than 60% of seropositive cattle for slaughter. Furthermore, failure to replace animals lost to the study was due to owners not agreeing to recruit new animals especially during the dry season when they consider animals to have less blood due to shortage of feeds and water, and some were not willing to tag their animals.

Animals lost to the study were, however, followed up if they returned to the original herd or flock. A summary of number of animals sampled in each households per visit is shown in Table 6.1.

Table 6.1: The number of domestic ruminants sampled in each household at each visit.

Household ID	*Visit-1*	*Visit-2*	*Visit-3*	*Visit-4*	*Visit-5*
A	87	85	72	68	62
B	60	56	47	46	42
C	70	33	26	19	22
D	64	6	20	0	NA
E	79	58	54	61	NA
F	104	90	4	NA	NA

NA = Household not visited as a result of late recruitment or difficulties in locating herds during seasonal movements

0 = Household visited but no animals present due to seasonal movements

6.3.2 Incidence of brucellosis c-ELISA seropositivity

The incidences of brucellosis c-ELISA seropositivity between visits 1-2 and 2-3 were calculated on the basis of six households. Two households (ID D and F) were excluded in the calculations for incidences between visits 3-4 and 4-5 as some households were not visited or animals were not present. Also household ID E was not considered in the calculation for incidence between visits 4-5 as it was not visited in the final visit.

The incidence rate of brucellosis c-ELISA seropositivity between the first and second visits was 0.183(183/1000) cases per animal-3 months at risk, equivalent to 0.732 (732/1,000) cases per animal-year at risk. Excluding the beef ranch, the incidence of brucellosis c-ELISA seropositivity was lowered to 0.079 (79/1000) cases per animal-three months at risk, equivalent to 0.316 (316/1,000) cases per animal-year at risk. The incidence

rate varied depending on the number of domestic ruminants at risk and new cases at different visits (Table 6.2)

Table 6.2: The incidence of brucellosis c-ELISA seropositivity at three month intervals for twelve months period.

Visits	*Number of animals at risk at start*	*Number of animals at risk at the end*	*New cases in that period*	*Incidence*
1-2	327	186	47	0.183
2-3	186	156	7	0.041
3-4	156	157	3	0.019
4-5	106	102	2	0.019

Of 59 new cases, 76.3% (45) were cattle and 23.7% (14) were small ruminants. Ninety three percent of c-ELISA seropositive cattle were female. Sixty nine percent of c-ELISA seropositive small ruminants were female. There was variation between household incidences with higher incidence rates in households screened during the first three months (Table 6.3). Subsequent visits however, revealed a rapid decline in incidence in some households. There was a linear relationship between household seroprevalence and incidence of brucellosis c-ELISA seropositivity in the subsequent visit ($r = 0.93$). Households that had higher seroprevalence at baseline were observed to have a high incidence rate on the subsequent visit (Table 6.4).

Table 6.3: Household incidence by species at three month intervals for a twelve month period

Visit	*Household-A*		*Household-B*		*Household-C*		*Household-D*		*Household-E*		*Household-1*	
	Cattle	*S/R*	*Cattle*	*S/R*	*Cattle*	*S/R*	*Cattle*	*S/R*	*Cattle*	*S/R*	*Cattle*	*S/R*
1-2	0.027	0.22	0.083	0.095	0.09	NA	0.065	0	0	0.067	0.875	NA
2-3	0	0.043	0	0.05	0.05	NA	0	0.148	0.333	0	1	NA
3-4	0	0.043	0.043	0	0	NA	0.026	0	0	0	NV	NA
4-5	0.033	0	0	0	0.061	NA	NV	NV	NV	NV	NV	NA

NA =Small ruminants were not sampled

NV = Household was not visited

Table 6.4: The relationship between seroprevalence at the initial sampling point and incidence rate after three months in individual households

Household ID	*Initial seroprevalence in cattle (%)*	*Incidence in cattle*	*Initial seroprevalence in small ruminants (%)*	*Incidence in small ruminants*
A	12.2	0.027	38.5	0.22
B	34.4	0.083	16.7	0.095
C	25.4	0.090	NA	NA
D	12.5	0.067	0	0
E	24.1	0.065	4.8	0
F	69.2	0.875	NA	NA

6.3.3 Relationship between incidence rate of brucellosis c-ELISA seropositivity and seasonality

Households were visited during both the wet and dry seasons. The wet season started in December and ended in June, whereas the dry season started in July and ended in November. New cases were categorised by season of the year. Households D and F were not considered at this stage as they were not visited during both wet and dry seasons. For the purpose of interpretation, cases identified during September and December visits were grouped under "dry season" and those identified during March and June visits were categorised under "wet season". There were 28 new cases in the four households (Households A, B, C and E). Of 28 new cases, 61% were diagnosed during the wet season and 39% during the dry season (Table 6.5). There was a significant statistical association between brucellosis c-ELISA seroposivity incidence and seasonality (P = 0.018, df = 1, 95%CI =0.0093, 0.0733) with a greater incidence during the wet season when parturition rate was higher than in the dry season (Figures 6.1a, 6.1b).

Table 6.5: Number of new c-ELISA seropositive cases by season

Duration by months	***Numbers of animals at risk at start***	***Number of animals at risk at the end***	***New cases at that period***	***Incidence***
January-March	219	168	12	0.062
April-June	159	152	5	0.032
July-September	187	164	9	0.051
October-December	114	109	2	0.018

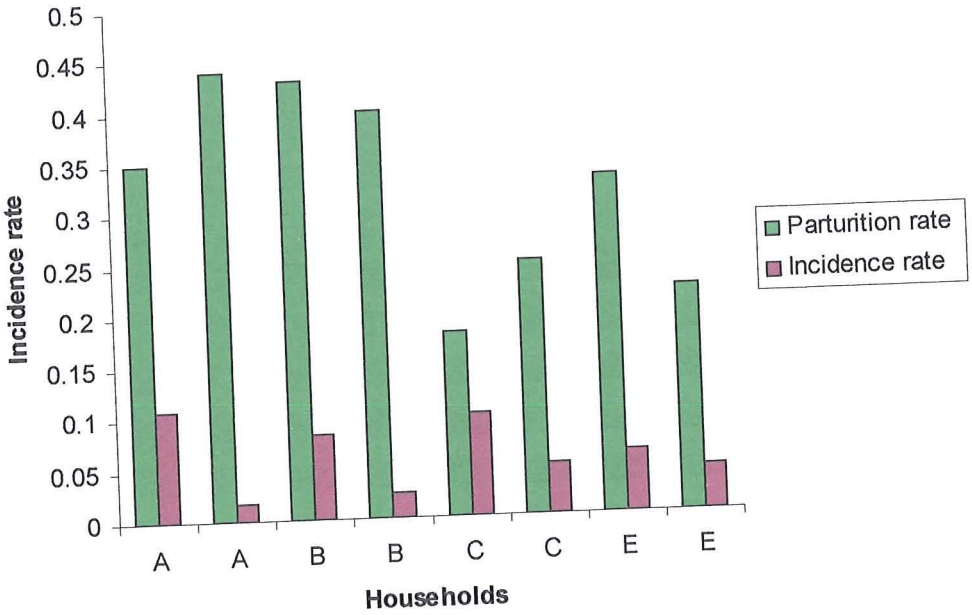


Figure 6.1(a): Relationship between incidence rate and parturition rate within households during the wet season

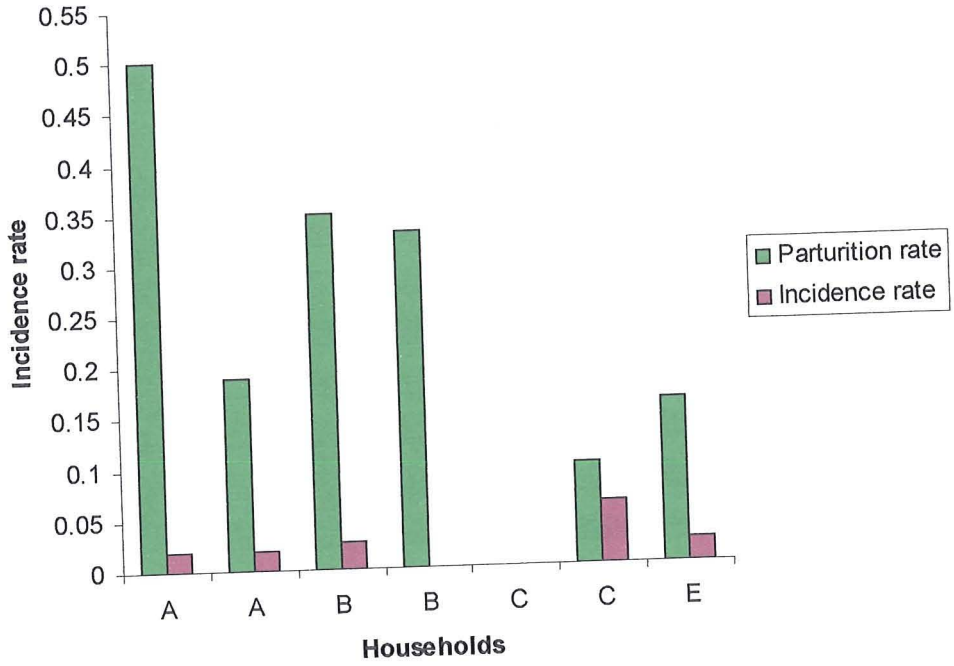


Figure 6.1(b): Relationship between incidence rate and parturition rate within households during the dry season

New c-ELISA seropositive cases diagnosed during the wet season among cattle and small ruminants were 60% and 54% respectively (Figure 6.2a, 6.2b). It was observed that one household (Household ID-A) had all new c-ELISA seropositive cases diagnosed in cattle during the dry season. The same household had the majority of new small ruminant c-ELISA seropositive cases diagnosed during the dry season (Figure 6.2b).

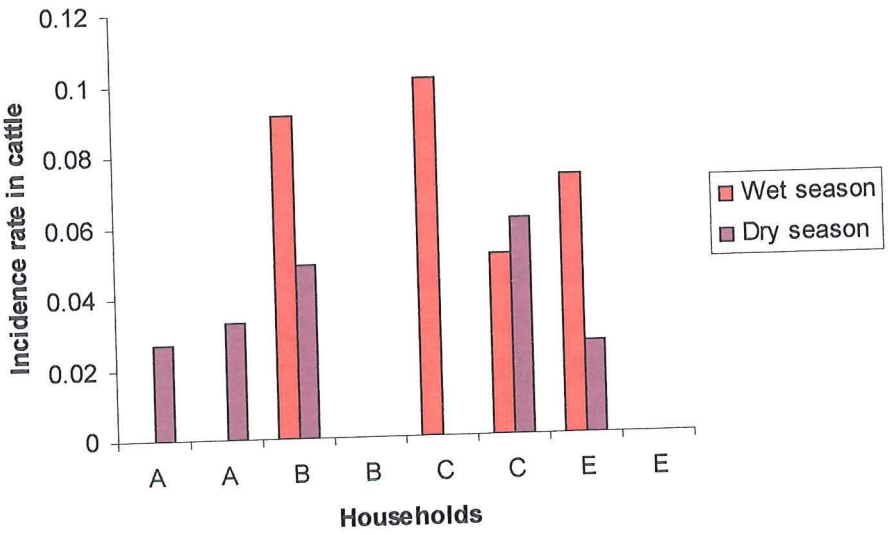


Figure 6.2(a): Relationship between cattle incidence rate and seasons within households

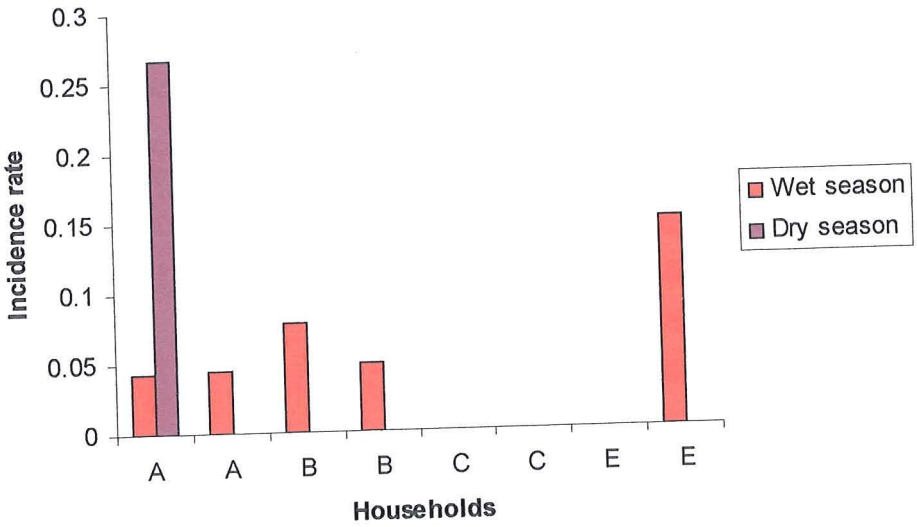


Figure 6.2b: Relationship between small ruminant incidence rate and seasons within households

From the current study it was observed that 16 female domestic ruminants (cattle, goats and sheep) showed positive to negative seroconversion. Fifty six percent of these animals had optic densities (ODs) of 50-60 c-ELISA classified as seropositive, which in the subsequent visits changed to ODs of 62-100 c-ELISA classified as seronegative. The remaining 44% of animals had ODs of 4-49 c-ELISA classified as seropositive, which in the subsequent visits changed to ODs of 67-83 c-ELISA classified as seronegative.

6.3.4 Survival probability of domestic ruminants at risk

The longitudinal life table was developed to estimate the probability of domestic ruminants remaining seronegative (surviving) within 3 months intervals, the probability of new cases of brucellosis and the probability of remaining seronegative from initial sampling to the end of the sampling period. The estimated probability of remaining seronegative for one year was 0.836 (Table 6.6).

Table 6.6: Longitudinal life table for brucellosis c-ELISA seropositivity for a twelve month period

Time (months)	*Animals at risk (n)*	*Seropositive animals (e)*	*Interval risk, q= e/n*	*Interval survival, p=1-q*	*Cumulative survival (s)*
0	Baseline				
3	128	13	0.102	0.898	0.898
6	101	0	0	1	0.898
9	96	4	0.042	0.958	0.861
12	102	3	0.029	0.971	0.836

Includes only animals observed for 12 months

6.3.5 Impact of brucellosis infection on production and reproduction

6.3.5.1 Reproductive assessment

A follow-up was undertaken on 210 mature female domestic ruminants over a period of twelve months in all five households. Among these, 26% had a history of previous abortion with small ruminants accounting for a high proportion (56%) of these cases. It was only possible to assess reproductive performance in 144 animals. This was due to a number of unavoidable issues, for example, lack of service records, inability to perform rectal or ultrasound examination for pregnancy detection and inconsistency of recollection of information by owners. During the period of twelve months of follow-up, 80 female domestic ruminants gave birth and 14 aborted (Table 6.7). Twenty five percent of domestic ruminants that gave birth were c-ELISA seropositive. Of the 94 female animals that were expected to parturate 15% aborted with 29% of the aborted animals being c-ELISA seropositive.

Table 6.7: Normal births, abortions and loss to the study in female domestic ruminants

Visits	*Normal births*	*Abortions*	*Pregnant animals lost to the study*	*Animals with no records lost to the study*
1-2	26	9	16	26
2-3	25	2	7	13
3-4	20	2	17	15
4-5	9	1	10	12
Total	80	14	50	66

Retained placenta was observed in 4.3% of the domestic ruminants. Of these 75% were cattle and 25% were small ruminants. Among cattle that had retained placenta, 67% were c-ELISA seropositive. The remaining 33% of cattle and all small ruminants with retained placenta were c-ELISA seronegative. These cases were handled by farmers themselves using antibiotics or local herbs. The cost of using antibiotics in treating retained placenta ranged from Tshs. 1000-4000 (US$ 1-4) with an average of Tshs. 2400 (US$2.4) per case.

6.3.5.2 Seroprevalence of brucellosis in calves

A total of 79 calves were screened and 21.5% were found to be c-ELISA seropositive. Forty seven percent of female calves were c-ELISA seropositive compared to 53% male calves. Of the c-ELISA seropositive calves, 82% were born from c-ELISA seropositive dams. One calf became c-ELISA seropositive three months after its dam had seroconverted. Twelve percent of the c-ELISA seropositive calves were born from c-ELISA seronegative dams. Of 62 c-ELISA seronegative calves, 21% were born from c-

ELISA seropositive dams. A significant statistical association was observed between serostatus in calves and dams (OR = 27, 95% CI = 5.46, 133.49), indicating that calves born from c-ELISA seropositive dams were 27 times more likely to be c-ELISA seropositive compared to calves born from seronegative dams.

The current study showed that six calves shown positive to negative seroconversion at different visits. For example, some calves had positive-negative-positive-negative or positive-positive-negative-positive c-ELISA serostatus during the period of one year.

6.3.5.3 The influence of dam's serostatus on calf growth rate

The current study showed that calves median heart girth was 94.5 cm for those were suckling from seropositive dams and 93 cm for those were suckling from seronegative dams.

Twenty eighty of the 79 calves had their heart girth measured three times at 3 monthly intervals. Seventy one percent of these calves with a median heart girth of 93 cm were suckling from c-ELISA seronegative dams and 29% of calves with a median heart girth of 94.5 cm were suckling from c-ELISA seropositive dams. Two groups of calves were formed based on this category and their measurements analysed. Using the Wilcoxon test, the lower sum of the heart girth ranks (97.4) lie between the critical value from the Wilcoxon table (77-155) based on the two groups ($n_1 = 8$, $n_2 = 20$) and thus the difference was not statistically significant ($P>0.05$).

6.4 Discussion

From the current study, the overall incidence of brucellosis in four households during the first three months interval was 0.183 resulting in an estimated 0.732 (732/1,000) cases per animal-year at risk. There was variation in the incidence of brucellosis c-ELISA seropositivity among the three months periods. Following the second visit, the incidence rate declined in the majority of households, suggesting some variation in risk factors on transmission rates existed in the area.

Over the period of twelve months, 59 new brucellosis c-ELISA seropositive cases were encountered. Of the new cases identified, 93% were female cattle and 69% were female small ruminants. A high proportion of females being c-ELISA seropositive could be due to the fact that females are more prone to *Brucella* infection compared to males based on their behaviour of licking each other after parturition. Furthermore, *Brucella* organisms have a special affinity for a sugar alcohol called erythritol present in the placenta of domestic ruminants. This sugar is elevated during pregnancy and stimulates growth of *Brucella* organisms following infection (Bishop, *et al.*, 1994). Therefore, the effect of erythritol in female animals could possibly be the cause of the difference in *Brucella* seropositivity or this could be due to other physiological mechanisms. Similar findings were observed during the cross-sectional study where females were more seropositive than males (Chapter V).

It was observed from the current study that a linear relationship existed between household baseline seroprevalence and the incidence of brucellosis c-ELISA seropositivity on the

subsequent visit. This could be explained by the fact that the higher the number of infected animals in the herd or flock, the higher the risk within the herd or flock. A similar study conducted by Lithg-Pereira *et al.*, (2004) found that flocks which delayed culling *Brucella* seropositive animals had more new brucellosis seropositive cases in the subsequent screening. The risk may be even higher if a large proportion of infected animals are reproductively mature females, as following parturition, they may spread the infection to animals at risk. This emphasizes that immediate culling of female positive reactors may be an important control measure to prevent further spread of infection between animals and subsequently to humans.

The current findings revealed that the incidence rate of infection was significantly higher during the wet season compared to the dry season. High numbers of new brucellosis c-ELISA seropositive cases during the wet season coincided with the high parturition rate during wet season. This could explain the high numbers of new brucellosis c-ELISA seropositive cases during this period as environmental contamination could be expected to be high through exposure to foetal fluids and placentae. Environmental contamination during the wet season may have a significant effect as it creates a favourable climate for *Brucella* organisms to survive longer thus providing more exposure time to animals at risk (Crawford, *et al.,* 1990; Bishop, *et al.*, 1994). Furthermore, congregation of animals especially females in the kraal facilitate licking each other after calving or abortion thus spreading the infection to animals at risk. This was supported by the cross-sectional findings (Chapter V) where calving in the kraal was associated with c-ELISA seropositivity. Further interesting results were observed in one household which had a higher incidence rate of brucellosis c-ELISA seropositive animals during the dry season.

There could be some risk factors within or between households that contribute to the perpetuation of infection during the dry season such as seropositive animals giving birth and spreading the infection among animals at risk in the household or those animals come into contact with contaminated flocks, herds or pastures.

The current study observed that 16 female domestic ruminants showed positive to negative seroconversion. Fifty six percent had ODs of 50-60 c-ELISA as seropositive and changed to ODs of 62-100 c-ELISA as seronegative. The variation observed was probably due to cross-reaction as majority had optic densities close to the cut-off (60). Certain bacterial organisms like *Yersinia enterocolitica* O:9, *Escherichia coli* O:157 and *Salmonella urbana* shared similar antigenic properties with *Brucella spp* that may result in such variations. For example, isolation of *E. coli* O:157 from cattle in Tanzania provided an evidence for possible cross-reaction with brucellosis serological tests (Hayghaimo, *et al.*, 2001). False positive reactor animals had also been reported elsewhere (Hilbink *et al.*, 1995; Weynants *et al.*, 1996; Pouillot *et al.*, 1997; Bercovich *et al.*, 1998). Therefore, animals that showed optic densities close to the cut-off should be considered as inconclusives and probably retested or otherwise tested for these cross-reacting organisms. Forty four percent of animals had ODs of 4-49 c-ELISA as seropositive and 66-83 c-ELISA as seronegative. A wide range observed in the ODs could be due to the fact that these animals succumb to infection but the infective dose was not enough to develop into disease (Bishop, *et al.*, 1994; MacMillan, Personal communication, 2004) or some other factors that were not measured in this study.

In addition, following exposure to *Brucella* infection a period is elapsed before the animal become serologically positive (lag-phase). Immunoglobulins M (IgM) become evident in the early stage of infection (Berman, 1981) followed by antibody of the IgG class (Elzer *et al.,* 1994) and both can be detected by c-ELISA technique. However, the c-ELISA antibody curve showed that titre levels differ at different stage of the disease with higher titres observed during acute stage and decline as the disease becomes chronic (Araj and Kaufmann, 1989). Therefore, apart from differences described above, some individual variations may exist based on their immune status and physiological status such as stage of pregnancy and following abortion (Bishop, *et al.*, 1994).

Results of the current study showed that 15% of animals had abortion and cost the livestock owner an average of Tsh.2400/= ($2.4) to attend each case of retained placenta. These observations were an indication that brucellosis attributed to abortions and retained placenta. This could be supported by the cross-sectional findings where 12% (Attributable risk) of abortions were attributed to brucellosis and an association between c-ELISA seropositivity and retained placenta was observed. In addition to calf loss and cost of treating retained placenta, brucellosis interferes with calving pattern and results in long calving intervals and impairs milk production. Although the impact of brucellosis on abortion may be confounded by other causes, any intervention will result in benefits such as increased number of replacement animals, reduced costs of treating retained placenta and ultimately preventing human infection. This is especially important in pastoral poor communities where livestock and livestock products are crucial for their livelihood and welfare.

There was a significant statistical association between c-ELISA seropositive calves and c-ELISA seropositive dams with calves born from seropositive dams being 27 times more likely to be infected compared to calves born from seronegative dams. A higher proportion of seropositive calves from seropositive dams as observed in this study may indicate that the source of infection could be either through uterine transmission or ingestion of contaminated colostrum or milk. Similar suggestions were put forward by others (Crawford *et al.*, 1986; Bishop, *et al.*, 1994) where transovarial transmission and ingestion of milk from infected dams were considered as the major sources of infection in calves. Calves that were c-ELISA seropositive while their dams were seronegative could be due to the fact that in the pastoral herds calves can suckle from different dams provided they are docile. Such practice of leaving calves suckling to other dams could be a means of transmitting brucellosis to calves within a herd. Other sources of infection could be through ingestion of contaminated pastures as some of calves graze on pastures nearby.

Furthermore, 21% of seronegative calves were born from seropositive dams. This could be explained by the fact that calves born from seropositive dams their antibodies fall to undetectable level probably due to failure of infection establishment (Nicoletti, 1990) or due to elimination of infection and return to seronegative status (Bishop, *et al.*, 1994). Although these calves were serologically negative, other studies have shown that they harbour the organisms as positive cultures following cultivation of tissues from seronegative calves were observed (Crawford, *et al.*, 1990). Another interesting finding from this study was the tendency of some calves to exhibit variation in serostatus at three months intervals. Such an observation made it difficult to ascertain the serostatus at the calfhood stage. Therefore, based on these observations calves born from positive dams

and those infected from other sources may be treated as suspicious regardless of their serostatus and should be excluded from breeding programmes as suggested by others (Cattlin and Sheehan, 1986).

There was no statistical significant difference in growth rate between calves suckling from seropositive and seronegative dams. Lack of significant differences could be because brucellosis has not caused significant effect on the milk yield. Also intervention by herd owners allowing calves to suckle from other dams when their dams have little milk affects this observation (Personal observation, 2003). The small size of the longitudinal study, especially in light of significant loss to follow-up meant that it was not possible to stratify the analyses to account for some possible confounders for the outcome variables investigated. These include variations in the ways calves were managed, breed variations and possible suckling of animals by children.

Therefore, it could be concluded from this study that the incidence rate in an individual household was mainly determined by the number of animals infected in the household, and the seasonal pattern observed could be useful in developing strategic control measures. Furthermore, the inconsistencies in serostatus observed in calves suggested future problems at herd level with replacements and in controlling the disease.

CHAPTER VII

7.0 EVALUATION OF DIAGNOSTIC TEST RESULTS

Abstract

The study was carried out to identify the current *Brucella species* present in the livestock population in Tanzania using bacteriological culture. In addition the Rose Bengal Plate Test (RBPT) was assessed based on the competitive enzyme-linked immunosorbent assay (c-ELISA) as a gold standard test. The agreement between the field RBPT (FRBPT) and laboratory RBPT (LRBPT) results was also assessed.

Of 142 milk samples cultured, only one milk sample was culture positive and the organism was identified as *Brucella melitensis* type-1. Blood (124) and placental (26) samples were all found to be culture negative. The positive milk sample was from a goat that was seropositive by both FRBPT and c-ELISA, had no history of abortion, and was kept together with cattle.

Using the c-ELISA as the gold standard the FRBPT had a diagnostic sensitivity and specificity of 42% and 98% and the positive and negative predictive values were 50% and 97% respectively. Adoption of a c-ELISA cut-off of 70% improved the sensitivity of the FRBPT when compared to the c-ELISA cut-off of 60% used in the study. The FRBPT performed with higher diagnostic sensitivity (45%) and specificity (96%) when testing serum samples from pastoral herds and flocks compared to serum samples from agro-

pastoral herds and flocks where the sensitivity was 13% and the specificity 100%. False negative animals were more frequently present in the agro-pastoral farming system (88%) than in the pastoral farming system (55%). Testing samples in the field and in the laboratory revealed that the FRBPT performed better than the LRBPT with a Youden's index of 0.4 for the FRBPT compared to a Youden's index of 0.3 for LRBPT. The agreement between the FRBPT and LRBPT was 0.6 (Cohen's kappa), indicating that the field and laboratory tests had moderately good agreement.

The findings indicated that *B. melitensis* is present in pastoral animals in Tanzania. The RBPT performed relatively poorly and the use of this test in pastoral and agro-pastoral animals and in humans in Tanzania needs further improvement.

7.1 Introduction

The current diagnosis of brucellosis is based on clinical observation that may be complemented by serology, microbiological culture and molecular techniques (Ariza, *et al.*, 1992; Baily, *et al.*, 1992; Romero, *et al.*, 1995). Diagnosis of brucellosis in animals is based on clinical signs such as abortion, which usually occurs in the third trimester of pregnancy, hygromas, retained placenta and infertility in females. In males, brucellosis is characterised by orchitis, epididymitis, hygromas and occasionally inflammation of the seminal vesicles (Radostits, *et al.*, 2000). However, none of these clinical signs are pathognomonic (Radostits, *et al.*, 2000). Bacteriological culture of *Brucella* organisms is a traditional technique which provides the most accurate method for identification of infection (Corbel, *et al.*, 1983). The World Health Organisation (WHO) (1997) and Office

International for Epizootics (OIE) (2004), recommended culture of *Brucella* species from several types of specimen including uterine discharges, aborted foeti, udder secretions, blood during the acute phase of the disease or selected tissues such as lymph nodes, testes and epididymes. However, the procedure is time consuming, laborious and poses considerable risk of infection to laboratory personnel (Baily, *et al.*, 1992; Corbel, 1997; Gallien, *et al.*, 1998).

Furthermore, several serological tests have been developed for the detection of antibodies to brucellosis including the Serum Agglutination Test (SAT), Rose Bengal Plate Test (RBPT), Complement Fixation Test (CFT), Milk Ring Test (MRT), Rivanol Precipitation Test (RvPT), Coomb's test (CT), Enzyme Linked-Immunosorbent Assay (ELISA) and Fluorescence Polarisation Assay (FPA) (Corbel, 1988; Bishop, *et al.*, 1994; Nielsen and Gall, 2001). The recent development of a Competitive ELISA (c-ELISA) has been useful to distinguish vaccinated and naturally infected animals (Biancifiori, *et al.*, 2000), has high power in discriminating cross-reacting organisms, may be used to test samples from different species simultaneously and has high sensitivity (95.2-99.4%) and specificity (98.9- 99.7%) (Biancifiori, *et al.*, 2000; McGiven, *et al.*, 2003). The FPA has not been widely used (McGiven, *et al.*, 2003), but has been found to be a simple and rapid technique with high sensitivity (99.1%) and specificity (99.6%), and it can be used in the field (Nielsen, *et a*l., 2002).

In Tanzania, the SAT has been used as a standard screening test in the serodiagnosis of brucellosis (Mahlau and Hammond, 1962; Mahlau, 1967; Staak and Protz, 1973; Kitaly, 1984; Jiwa *et al.*, 1996; Weinhaupl, *et al.*, 2000) even though it has some limitations

(Radostits, *et al.*, 2000). These limitations include false positives in vaccinated animals, cross reactions with other gram negative organisms, and false negatives in chronic infections, in the early incubation period, or in recently aborted cases (Corbel, 1988; Bishop, *et al.*, 1994; Radostits, *et al.*, 2000). The RBPT has also been used in several studies in Tanzania for screening purposes (Kagumba and Nandokha, 1978; Otaru, 1985; Swai, 1997; Niwael, 2001; Minja, 2002) but its performance in Tanzania had never been evaluated. Both the RBPT and the SAT are simple and easy to perform, however the SAT requires basic laboratory equipment whereas the RBPT is a spot agglutination test that does not require any laboratory equipment (Macmillan, 1990) and is therefore easier to perform in the field (Baum, *et al.*, 1995). Although the low pH (3.6) of the Rose Bengal antigen enhances its specificity, the temperature of the antigen and the ambient temperatures at which the reaction takes place may influence the sensitivity and specificity of the test (Macmillan, 1990).

The introduction of newer diagnostic techniques in Tanzania has been difficult due to the cost involved and lack of government commitment to controlling brucellosis. Following the occurrence of human brucellosis in pastoral and agro-pastoral communities (Minja, 2001; Niwael, 2002; Kunda *et al.*, 2004), the use of appropriate tests is crucial for both livestock and human health as these would facilitate immediate and appropriate decision making, especially so in the management of human cases and in controlling brucellosis in livestock. A more robust diagnostic test than the SAT is required in livestock so that potential sources of infection to humans can be identified and controlled. The choice of a diagnostic test for brucellosis diagnosis in livestock may require consideration of the prevalence of infection in the population, the purpose of testing and economic implications

(Greiner and Gardner, 2000). There may be different requirements of diagnostic tests in different situations. For example in farming systems with low disease prevalence such as the agro-pastoral farming systems (chapter V), even a test with high sensitivity and specificity results in a low positive predictive value (Greiner and Gardner, 2000).

In populations with high disease prevalence a diagnostic test with high sensitivity may initially be required to identify positive animals. However, as the disease prevalence declines a test with high sensitivity and specificity will be required (Thrusfield, 1995).

It is the purpose of this study to evaluate the performance of RBPT as a field and as a laboratory screening test. Identifying the existing *Brucella* species present in different animal species in Tanzania is important as this has not been attempted in the majority of previous studies.

Therefore, this study aims to:

(i) Determine the *Brucella species* currently present in Tanzania.

(ii) Evaluate the RBPT as a field and laboratory test in Tanzania

7.2 Materials and methods

7.2.1 Study area

The study area and farming systems have been described in detail in Chapter II section 2.1.

7.2.2 Cultivation of Brucella organisms

During the cross-sectional study, samples for culture were collected from domestic ruminants. Blood samples from FRBPT positive animals were collected and retained for culture while milk and placenta samples were collected regardless of animal serostatus and kept for culture as described in Chapter II. However, out of 26 placenta samples collected two were from aborted cows. A total of 375 samples were collected for culture during the study (Table 7.1).

Table 7.1: Distribution of samples for culture by species

Samples/Species	*Cattle*	*Small ruminants*	*Total*
Blood	80	100	180
Milk	110	59	169
Placentae	26	0	26

Culture, identification and typing were carried out according to the protocol of Corbel *et al* (1983). The samples were cultured on the Farrell's modified serum dextrose agar as a selective media for *Brucella* organism growth. Antibiotics and antimicrobial agents were added to the media to suppress fast growing organisms. The Serum Dextrose Agar (SDA) was used for sub-culture. All cultures were performed at the Veterinary Laboratory Agency (VLA), UK.

7.2.3 Animal sampling, samples collection and serology

Sampling procedures and sample collection were described in detail in Chapter II. The Rose Bengal Plate Test (RBPT) antigen used in the study was stained *Brucella abortus* antigen donated by VLA Weybridge, UK (batch numbers 269 and SG276). Field and laboratory RBPT were carried out as described in Chapter II section 2.7.1. During the cross-sectional study, (Chapter V) the FRBPT was used to analyse 3561 livestock sera in the field and feedback on the results was given to respective household owners. Among these samples, 2032 were livestock sera from pastoral areas whereas 1529 were from agro-pastoral areas. These samples were later transported to Sokoine University where the LRBPT was performed. These samples were processed as described in Chapter II and sent to VLA, UK for the c-ELISA test. The c-ELISA was considered as a gold standard test with the Optical Density (OD) cut-off at 60%. Any sample with OD $\leq$ 60% was considered to be positive and OD > 60% was considered to be negative.

7.2.4 Data analysis

Data were handled using Microsoft Excel 97 spread sheet. The diagnostic sensitivities, diagnostic specificities, positive and negative predictive values were calculated according to Thrusfield (1995). The overall measure of sensitivity and specificity of a test was determined by calculating Youden's index. The agreement between FRBPT and LRBPT was determined using Cohen's kappa (Woodward, 2005). The Youden's index (Y) is given by **(Se+Sp)-1** where Se = sensitivity and Sp = specificity. The high value of Y is considered optimal. Cohen's kappa is given by **(Σa-ΣEf)/N- ΣEf)** where Σa is the total

number of agreements by summing the values in the diagonal cells (true positives and true negatives), ΣEf is the sum of the expected frequencies for the number of agreement that would have been expected by chance and applied only to the diagonal cells and N is the total number of observations. True prevalence (TP) of disease was estimated by using the sensitivity and specificity of ELISA test given by, $\mathbf{TP = (P^T + Sp)-1/(Se+Sp)-1}$, where P^T = test prevalence.

7.3 Results

7.3.1 Cultivation of Brucella organisms

Of the 375 samples collected 292 were cultured and 83 (56 blood, 27 milk samples) were not cultured. Milk samples were unsuitable for culture because of being soured and the remaining 56 missing blood samples were lost. Of the samples cultured, only one (0.7%) milk sample was found to be positive (Table 7.2). Following identification and typing, the organism was identified as *Brucella melitensis* type-1. The positive milk sample was collected from a goat that was positive by both the FRBPT and c-ELISA. Further information indicated that the goat had delivered twice with no history of abortion but had experienced retained placenta on one occasion.

Table 7.2: Bacteriological culture results

Samples	*Positive*	*Negative*	*Total*
Blood	0	124	124
Milk	1	141	142
Placenta	0	26	26
Total	1	291	292

7.3.2 Evaluation of the Rose Bengal plate test

During the cross-sectional survey, 3386 out of 3561 livestock serum samples were screened using the FRBPT and c-ELISA as a gold standard test (Table 7.3). However, 175 serum samples were not analysed by c-ELISA due to damage during transportation or misidentification.

Table 7.3: Cross-tabulation of FRBPT and c-ELISA seropositivity results

FRBPT	*c-ELISA (standard)*					
	Positive	Negative	Total	Sensitivity (%)	Specificity (%)	Positive predictive value (%)
Positive	75	76	151	42.1	97.6	49.7
Negative	103	3132	3235			
Total	178	3208	3386			

The diagnostic sensitivity and specificity of FRBPT were 42.1% and 97.6% respectively (Table 7.3). The positive predictive value was 49.7%. The Youden's index was 0.39. The performance of the FRBPT was re-calculated using different c-ELISA cut-offs to observe

any possible agreement in test performance. The FRBPT agreement was improved when the c-ELISA cut-off was set at 70 rather than 60 (Table 7.4).

Table 7.4: The agreement between FRBPT and c-ELISA using different c-ELISA cut-offs

C-ELISA cut-offs	*Sensitivity(%)*	*Specificity(%)*	*Positive Predictive value(%)*	*Youden's index*
40	44.0	97.0	33	0.41
50	43.8	96.7	42	0.41
60	42	97.6	50	0.4
70	57.6	97.8	54	0.55
80	26.3	98.1	60	0.24

When serum samples from pastoral and agro-pastoral households were analysed separately agro-pastoral serum samples gave diagnostic sensitivity and positive predictive values that were lower than the pastoral samples (Table 7.5). The estimated true seroprevalence based on c-ELISA were 10.2% and 7.3% compared to test seroprevalence of 8.3% and 1.1% in the pastoral and agro-pastoral farming systems respectively (Table 7.5).

Table 7.5: The performance of FRBPT using pastoral and agro-pastoral livestock sera with c-ELISA as a gold standard

Farming system/ Parameters	*Samples tested*	*Samples positive to FRBPT*	*Samples positive to c-ELISA*	*Samples positive to both tests*	*Sensitivity (%)*	*Specificity (%)*	*Positive Predictive Value (%)*
Pastoral	1949	146	162	73	45.1	95.9	50
Agro-pastoral	1437	5	16	2	12.5	99.8	40

Following LRBPT screening only 50 serum samples were positive by both the LRBPT and c-ELISA (Table 7.6).

Table 7.6: Cross-tabulation of LRBPT and c-ELISA seropositivity results

LRBPT	*c-ELISA (standard)*					
	Positive	Negative	Total	Sensitivity (%)	Specificity (%)	Positive predictive value (%)
Positive	50	48	98	28.74	97.52	51.0
Negative	124	3066	3190			
Total	174	3144	3288			

The diagnostic sensitivity and specificity of the LRBPT were 29% and 98% whereas the positive predictive value was 51%respectively (Table 7.6). The Youden's index was 0.3. When the performance of the FRBPT and LRBPT was compared, 74 livestock sera were seropositive in both field and laboratory settings. The agreement between the field and laboratory settings was 60% (Cohen's kappa-κ) (Table 7.7)

Table 7.7: Cross-tabulation of LRBPT and FRBPT seropositivity results

	FRBPT		
LRBPT	Positive	Negative	Total
Positive	74	26	100
Negative	78	3249	3327
Total	152	3275	3427

7.4 Discussion

Blood and placental samples were all found to be negative on culture. Only one of 142 milk samples was culture positive and the isolate was identified as *Brucella melitensis* biotype-1. Other studies have shown that culture techniques are more sensitive than conventional serological tests (Ferris, *et al.*, 1995). In contrast Baily, *et al.*, (1992) and Nimri, (2003) observed culture as being insensitive during the chronic stage of the disease and in treated human cases. The low isolation rate observed in milk samples in this study could be due to the fact that not all milk samples were collected from c-ELISA seropositive animals. More than 40% of blood samples collected were from c-ELISA seropositive animals. Failure to culture *Brucella* organisms could be explained by the fact that animals at the chronic stage of the disease, bacteraemia becomes intermittent thus circulating organisms are below the number that could give a positive culture. Similar observations were reported by Jubb, *et al.*, (1991) where bacteraemia was shown to occur at parturition. Frequent use of antibiotics to treat animals for prevalent diseases could contribute to the high level of negative cultures (Shirima personal observation, 2003). A

study conducted in humans suggested that for culture results, blood should be obtained from untreated individuals (Nimri, 2003). It is possible that storage and transport of samples varied and that some were not adequately and consistently controlled and thus this could influence the culture positivity. Hence lack of isolating *Brucella* organisms from samples obtained from seropositive animals does not exclude the possibility that animal products such as milk and blood may still be a risk to humans if consumed raw.

The development of molecular techniques such as the Polymerase Chain Reaction (PCR) has improved diagnosis of many diseases including brucellosis in both livestock and humans (Romero, *et al.*, 1995; Gallien, *et al.*, 1998). The PCR has been shown to increase sensitivity and specificity in the diagnosis of brucellosis (Fekete, *et al.*, 1992; Gallien *et al.*, 1998). The technique is less affected by the number of organisms present in the sample than culture techniques. The PCR can diagnose the disease in samples with less than 5 organisms by using fluorescence-labelled primers (Liu, *et al.,* 2001). The PCR is not limited by the stage of the disease or by cross-reactions (Baily, *et al.*, 1992; Gallien, *et al.*, 1998). Therefore, the development of a molecular technique could be helpful to overcome the problems of sensitivity associated with culture methodology.

The current isolation of *B. melitensis* biotype-1 was comparable to the study conducted by Schiemann and Staak (1971) and Mahlau (1967) in Tanzania where *B. melitensis* was isolated from wild animals, goats and humans. Also *B. melitensis* has been isolated in domestic animals and humans in Mediteranean countries and China (Refai, *et al.*, 2002; Shang, *et al.*, 2002). The problem of *B. melitensis* in Tanzania poses risks to humans who consume goat's milk and handle or assist parturitions. The disease is more severe in

humans compared to *B. abortus* infection (OIE, 2004) and has a high morbidity. Keeping cattle and small ruminants together in pastoral communities facilitates cross-infection which ultimately predisposes large populations of milk consumers and animal handlers to a high risk of *B. melitensis* infection. Therefore, the combination of managerial strategies and use of Rev. 1 vaccine against *B. melitensis* could be an appropriate in the control of *B. melitensis* infection.

The overall diagnostic sensitivity of FRBPT was lower (42%) when compared with other studies elsewhere (Baum, *et al.*, 1995; Martin-Moreno, *et al.*, (1992) and the specificity (98%) was higher than that reported by Martin-Moreno, *et al.*, 1992). These variations could be attributed to the differences in the reference populations, sampling strategies, technical variations of the test characteristics and handling of intermediate results (Greiner and Gardner, 2000). In the current study, it was most likely that ambient temperatures in the field environment could have influenced the FRBPT. Ambient temperature was observed to vary such that in some occasions small ruminants' sera coagulated following centrifugation due to cold weather (Personal observation, 2003). Also during cloudy weather, natural light was poor making examination of agglutinates difficult. This may result in false negatives and explain the low sensitivity of the FRBPT. The cut-off value for c-ELISA was set at 60% (OD) to ensure a higher sensitivity and specificity with limited non-specific reactions in a low disease prevalence setting such as Europe (MacMillan, Personal communication, 2005). It was anticipated that the low sensitivity of RBPT was associated with this cut-off value but re-analysis at different cut-off points showed that at the cut-off value of 70 the sensitivity of the FRBPT was increased from 42% to 58% without affecting the specificity. However the low FRBPT sensitivity was

unexpected and therefore other factors may have attributed to this low sensitivity or the Rose Bengal antigen may be genuinely insensitive. Appropriate cut-off values for c-ELISA in Tanzania are necessary as the current cut-off value showed big discrepancies between test seroprevalence (1.1%) and estimated true seroprevalence (7.3%) in the agro-pastoral livestock population.

The different sensitivity of the FRBPT observed between results from agro-pastoral and pastoral livestock populations could be due to high number of false negatives (88%) in agro-pastoral animals compared to pastoral animals (55%). Therefore, it is suggested that in areas with low seroprevalence at least two screening tests should be used to increase the likelihood of identifying infected animals (Pouillot, *et al.*, 1997) in the absence of a gold standard test.

CHAPTER VIII

8.0 GENERAL DISCUSSION

8.1 Introduction

In 1994/95 the national sampling study estimated that there were 15.6 million cattle, 10.7 million goats and 3.5 million sheep in Tanzania (Ministry of Agriculture and Co-operatives (MAC), 1997). Approximately 98% of these animals are kept in pastoral and agro-pastoral farming systems. Although the sector faced several constraints in realising its potential, the sector contributed 18% of the national Gross Domestic Product (GDP) (MAC, 1997). According to the MAC the major constraints identified included inadequate and poor nutrition and water sources, low genetic potential of indigenous cattle and small ruminants, inadequate and poor animal health services and infrastructures, poor and inadequate marketing and processing infrastructures, high prevalence of diseases such as tick borne diseases, trypanosomosis, parasites, zoonotic diseases and lack of guaranteed security of land tenure. In communal grazed areas, land ownership is still uncertain and increasing social conflicts between livestock owners and crop producers are prevalent. The livestock sector therefore can not maximise its production due to the aforementioned problems. Several zoonotic diseases are either only partially controlled or in some cases, no control measures are in place. This may be attributed to lack of resources, lack of data to justify control measures, and lack of awareness among communities, experts and policy makers. The information generated from this current study on the magnitude and impact of

brucellosis in livestock keeping communities may help to develop logical evaluation and possible interventions in future.

8.2 Major findings

The major goal of this thesis was to assess community knowledge, awareness and practices on zoonoses in various livestock production systems, to gather baseline information on brucellosis in livestock and wildlife in Tanzania, to determine brucellosis seroprevalence in domestic ruminants and humans and identify risk factors associated with livestock seropositivity, to assess brucellosis dynamics and impact on livestock production and reproduction, and to identify *Brucella* species infecting livestock in Tanzania and evaluate the performance of RBPT as a field test in Tanzania.

An assessment on knowledge, awareness and practices of zoonoses in livestock keeping communities was undertaken. There were no previously published studies to assess these variables in Tanzania and this study was probably the first attempt to collect this information in various farming systems in this country. Of the zoonotic conditions identified during the PRA survey, 63% were zoonotic according to WHO (1959) definitions. Identification of zoonoses by livestock keepers was variable with the most common conditions reported as rabies, tuberculosis, brucellosis and anthrax. Knowledge of brucellosis among respondents was identified in both the PRA and cross-sectional surveys. During the PRA survey, only cattle were linked with brucellosis, whereas in the cross-sectional study, all domestic ruminants were identified as being associated with brucellosis. Although pigs are reared in agro-pastoral communities, none of the

respondents during the PRA or the cross-sectional survey identified pigs as being associated with brucellosis. Pigs were not considered as important as other domestic animals in association with brucellosis.

None of the village respondents identified the clinical signs of brucellosis in animals during the PRA survey and only a limited number did so during the cross-sectional study. Failure of respondents to identify clinical signs has also been acknowledged in several veterinary texts (Radostits, *et al.*, 2000), as clinical diagnosis of brucellosis is considered to be generally difficult and clinical signs non-specific. The clinical signs of brucellosis are variable depending on the immune status of the herd (Radostits, *et al.,* 2000) and in newly infected herds frequent abortion after the 5^{th} month of pregnancy may be a cardinal feature. In areas where other conditions cause abortion at the same stage of gestation, further tests are required to confirm diagnosis of brucellosis. For example in Tanzania, tick borne diseases, trypanosomosis and stress may cause abortion at any stage of gestation.

All village respondents during the PRA identified ingestion as the principal route of transmitting brucellosis to humans, whereas during the cross-sectional study, a small proportion of respondents additionally identified contact during abortion and slaughter as a means of transmitting brucellosis to humans. The high proportion of respondents from both the PRA and cross-sectional survey who identified ingestion as a major route of transmitting brucellosis to humans, could be due to the eating habits such as drinking raw milk especially as sour milk, raw blood and consumption of certain internal organs while raw as described in Chapter III. Although during the cross-sectional survey only a small proportion of respondents identified contact as a means of contracting brucellosis, all

activities that predispose people to brucellosis are performed by family members without any protective materials. Whilst a high proportion of respondents were known to boil milk and cook meat as a means of preventing animal-derived diseases, direct contact by assisting at parturition and handling infected materials may be an important route which has not yet appreciated by livestock keepers. For example, an interesting observation in pastoral communities was the practice of using the mouth to aspirate foetal fluids to clear the nostrils in new born calves (Shirima, Personal observation 2003). Based on the three farming systems studied, pastoral farming communities are at greater risk compared to agro-pastoral and smallholder communities, (Chapter III; Chapter V) possibly due to large herds which results to increased contamination and thus may exacerbate the situation that predispose family members to infection.

The differences observed between farming systems on knowledge and awareness to various zoonoses could be useful in identifying the gaps that need to be addressed during public health education. For example emphasising small ruminants and pigs as domestic animals that can transmit brucellosis to humans and the importance of contact as a means of acquiring zoonoses. *Brucella melitensis* in humans is the most pathogenic organism among the other species to infect humans (WHO, 1997). Isolating *B. melitensis* from goats' milk in the study area (Chapter V) is evidence that the community is at risk of acquiring the infection and were unaware of the disease in small ruminants. Small ruminants could also maintain the disease in the cattle population as they are kept together. In addition, *Brucella suis* infection in pigs has shown to infect other domestic ruminants and thus constitute both veterinary and public health problems (WHO, 1997). Therefore,

small ruminants and pigs are important in the epidemiology of *B. melitensis* and *B. suis* in domestic animal populations.

These farming systems were studied further using stored sera to assess the seroprevalence of brucellosis and the species affected. Wildlife sera were also tested as interactions between livestock and wildlife animals exist in some areas, and the results of this part of the study are described in Chapter 4. These results indicated that brucellosis infection was present in the pastoral, agro-pastoral and smallholder dairy systems, albeit at variable seroprevalences. A higher seroprevalence was observed in pastoral, followed by agro-pastoral, with the lowest seroprevalence in the smallholder dairy farming systems. The seroprevalence difference observed between farming systems may be due to different management styles such as grazing pattern, herd size, frequency of introduction of new animals, and general hygiene. Among the wildlife animals screened, seropositives were detected in wildebeest, impala and buffalo. Buffalo had the highest seroprevalence (28%) among the seropositive wildlife animals (Chapter IV). The declining number of buffalo population in the Ngorongoro-Serengeti ecosystem in recent years (Tanzania Wildlife Conservation and Monitoring, 2004) may be attributed to brucellosis resulting from abortions and consequently lower numbers of replacement calves. However, this may need further study and comparison with other areas affected by the disease. Cross-transmission is possible in areas where domestic ruminants and buffalo share grazing pastures and water, as the presence of wildlife animals in these areas were positively associated with c-ELISA seropositivity in domestic ruminants (Chapter V). Therefore, the presence of brucellosis in both domestic and wildlife animals emphasizes the need for collaboration between livestock owners, livestock experts and wildlife personnel to formulate control

strategies especially in areas where they shared resources as observed in pastoral communities in the Ngorongoro district. A brucellosis task force involving livestock owners, veterinarians and wildlife experts was observed to perform well in brucellosis control in the Great Yellowstone areas, USA (Thorne, 2004 unpublished). Controlling brucellosis in the wildlife population may be practically difficult but in domestic ruminants it is feasible. Therefore, the plan would focus on the control of brucellosis within livestock population by vaccination, gradual culling of infected animals and improved hygiene. In addition, identifying risk factors, mapping the transmission trend and continuous education are important to enhance collaboration towards control and eradication of brucellosis. This will prevent possible cross-transmission of brucellosis between domestic and wildlife animals and future conflicts that may arise between livestock keepers and wildlife experts.

The intensification of the smallholder dairy sector in urban and peri-urban areas, involves the purchase of animals from different sources, which may result in changes to the magnitude of brucellosis seroprevalence observed in this study as no control strategy is currently in place. This may have a subsequent effect on human health, especially livestock keepers and milk consumers. The smallholder dairy sector is labour intensive and the inputs are higher than in other farming systems in Tanzania, therefore, introduction of a disease like brucellosis that impairs production and reproduction may potentially result into a significant economic loss to livestock keepers. Thus to protect against spread of *Brucella* infection in the smallholder dairy sector, strategic control measures should be formulated by livestock keepers and the local veterinary authorities. For example vigilant surveillance to detect the existing foci of infection and introduction of new animals may require veterinary attention. This includes herd identification through milk ring test (MRT)

and thereafter serological screening to identify individual reactors. A confirmatory test should be used and positive reactors culled. This will be possible through public health education and good collaboration with livestock owners. Although heifers may test negative to *Brucella* infection before being introduced into the herd, isolation during calving and post-calving screening is required as some serocovert after calving (Bishop, *et al.*, 1994; Radostits, *et al.*, 2000). It is possible to establish seronegative herds in the smallholder dairy if herd owners are co-operative and comply with experts' advice (Tungaraza, Personal communication 2002).

In Chapter V, the pastoral and agro-pastoral farming systems were studied further to explore risk factors responsible for brucellosis in livestock as these farming systems were found to have a high brucellosis seroprevalence during the baseline serosurvey (Chapter IV). Brucellosis infection was detected in both farming systems during the cross-sectional survey. All domestic ruminants were infected, with small ruminants having a higher proportion of c-ELISA seropositivity (5.8%) than cattle (4.7%). This suggested that for any future study and interventions, small ruminants should be given priority as they share all resources with cattle.

The long inter-calving intervals contribute to low productivity in domestic ruminants in Tanzania and were attributed to several factors including disease, nutrition and poor breeding regimens. The long calving, kidding and lambing intervals observed in this study were not statistically associated with c-ELISA seropositivity. Lack of association may be confounded by the fact that owners have a breeding regimen that resulted in long parturition intervals. The long calving intervals observed in the current study were smaller

than that reported by Swai (1997) in the smallholder dairy in the eastern zone of Tanzania, but higher than the recommended interval for Zebu which was 12-13.5 months (Rege, *et al.*, 2001). The use of plastic and skin sheaths in rams and bucks in an attempt to control breeding may have contributed to lack of association between lambing and kidding interval and seropositivity in small ruminants. Furthermore, lack of pastures and water especially during dry season with various stress factors may influence the reproductive cycle of these animals where the resultant effect masks the association between brucellosis infection and parturition intervals. A similar study conducted by Mokantla and colleagues (2004) in communal grazing areas in South Africa observed that sub-fertility of bulls and poor feeding management played a more important role in reducing pregnancy rate than brucellosis and other infectious diseases in cattle.

Chapter V differentiated the findings from the pastoral and agro-pastoral farming systems and the differences observed were due to the nature of the two farming systems as described in Chapter II section 2.1. The risk factors analysed were attributed to the differences observed in Table 8.1

Table 8.1: The summary results of c-ELISA brucellosis seropositivity in the pastoral and agro-pastoral farming systems

Pastoral farming systems	*Agro-pastoral farming systems*
Out of 30 herds 67% were seropositive	Out of 72 herds 7% were seropositive
Out of 26 flocks 65% were seropositive	Out of 63 flocks 14% were seropositive
Among cattle, 7.3% were seropositive	Among cattle, 1.1% were seropositive
Among goats, 9.7% were seropositive	Among goats, 1.5% were seropositive
Among sheep, 8.3% were seropositive	Among sheep, 1.2% were seropositive
There was no statistically significant difference between female and male c-ELISA seropositivity (P>0.05) in both cattle and small ruminants although females had a high proportion of infection	There was no statistically significant difference between female and male c-ELISA seropositivity (P>0.05) in both cattle and small ruminants although females had a high proportion of infection
A statistical association was observed between c-ELISA seropositivity and abortion in Cattle and small ruminants (OR = 5 in cattle and 2 in small ruminants)	There was no animal had history of abortion and being c-ELISA seropositive
There was no statistical association between households with a history of abortion and c-ELISA seropositivity (P>0.05)	There was no statistical association between households with a history of abortion and c-ELISA seropositivity (P>0.05)
There was a statistical association between female cattle with a history of retained placenta and c-ELISA seropositivity (P<0.01). The difference was not observed in small ruminant females	There was no individual female animal or household with a history of retained placenta and being c-ELISA seropositive
There was a statistical association between households with history of retained placenta and c-ELISA seropositivity (OR = 58)	

There was a statistically significant difference between age and c-ELISA seropositivity with high infection in older cattle compared to young animals (P<0.01). The difference was not observed in small ruminants (P>0.05)	There was a statistically significant difference between age and c-ELISA seropositivity in both cattle and small ruminants with high infection in older animals compared to young ones (P<0.05)
There was a statistically significant difference between herds/flocks and c-ELISA seropositivity (P<0.01) with infection being higher in big herds and flocks	There was a statistically significant difference between herd size and c-ELISA seropositivity (P<0.01) with more infection in big herds. The difference was not observed in flocks (P>0.05)
Herds and flocks size >80 are more likely to be seropositive (Figure 5.2)	Limited by the herd and flock size where the maximum limit was 60 animals
Seropositivity variation between households was 1-30% with a mean of 8.3% and variance of 64.3%	Seropositivity variation between households was 1-14% with a mean of 5.2% and variance of 19.1%.

Univariate analysis (Chapter V) showed that majority of risk factors were those related to management practices and were positively associated with c-ELISA seropositivity. However calving inside and taking manure outside for drying and used as bedding were associated with reduced c-ELISA seropositivity. Small herds and flocks, limited grazing areas that resulted in less interaction between herds, flocks and wildlife animals and infrequent introduction of new animals were risk factors associated with the reduced c-ELISA seropositivity in agro-pastoral farming system (Table 8.1). Small herds and flocks and, limited interaction with other herds were observed elsewhere to be associated with low seroprevalence of brucellosis in agro-pastoral farming systems (Kadohira, *et al.*, 1997; McDermott and Arimi, 2002). Large herds and flocks, interaction with other herds and

wildlife in grazing areas, calving in the boma and grazing areas, frequent introduction of new animals and dogs fed with aborted foeti and placentae were positively associated with increased c-ELISA seropositivity in pastoral farming systems (Table 8.1). Some of these risk factors were also suggested by Forbes, (1990), Bishop *et al.,* (1994) and Kadohira, *et al.,* (1997) to be associated with increased risk of brucellosis in livestock. Vaccination of cattle and small ruminants using S-19 and Rev-1 vaccines protect non-infected animals within infected herds (Radostits *et al.*, 2000). In addition, gradual culling of positive reactors, practice of good hygiene especially during calving and proper disposal of aborted foeti and placentae are important in the control of brucellosis. Such interventions may benefit human health and welfare by reducing the animal reservoirs and losses.

During multivariate analysis, three risk factors explained the final model for brucellosis c-ELISA seropositivity. The practice of feeding dogs with aborted foeti and placenta was positively correlated with c-ELISA seropositivity. However, aborted foeti were common in seropositive herds and flocks and likely to be fed to dogs, thus playing a key role in the epidemiology of brucellosis in the study area. Feeding dogs with aborted foeti was reported as a means of disposal during the PRA study (Chapter III). Also during the PRA proper disposal of aborted materials was only reported in majority in the smallholder households with only a few owners in the agro-pastoral households burying them. In the pastoral farming system however, late aborted foeti were consumed in some households otherwise together with placentae, they were frequently thrown to dogs as a means of disposing of them (Chapter III). The role of dogs in brucellosis dissemination was demonstrated by Forbes (1990) where outbreak of brucellosis in cattle occurred in a farm with infected dogs. In this study the role of dogs was probably through contaminating the

environment by carrying pieces of placenta and aborted foeti from one place to another. As this was one of the major risk factor for brucellosis c-ELISA seropositivity, community education and awareness to discourage such practices and to devise appropriate means of disposal is important. From Table 8.1, the agro-pastoral farming system was associated with a lower risk for brucellosis in the final model that resulted in infection being approximately 7 times less compared to pastoral farming system. Small herds and flocks, limited grazing with less interaction and general hygiene may have attributed to low seroprevalence in the agro-pastoral farming system (Chapter V).

The spatial distribution of c-ELISA seropositive households showed that households within pastoral and agro-pastoral farming systems assumed a contagious distribution (Thrusfield, 1995) where in both systems the variance was greater than the mean (Table 8.1). However, spatial clustering was higher in the pastoral households than in agro-pastoral households. Such variation suggests that the transmission rate varied between households within the farming system and is important in identifying the introduction of the disease (Thrusfield, 1995) within households. Risk factors within households may be of greater importance compared to risk factors between households.

The relationship between families' seropositivity and their herds was observed in the current study with families in the infected herds being 3 times more likely to be seropositive than families in the seronegative livestock households. Families with the highest c-ELISA seropositivity were observed in Ngorongoro district which is a pastoral district, followed by families in other districts which are predominantly agro-pastoralist. Babati district had no families with brucellosis and there were no seropositive herds or

flocks in this district. Absence of seropositive families in Babati district was supported by the fact that human brucellosis usually occurred when brucellosis was present in livestock populations in the area (Alausa, 1980). However, absence of infection in both livestock and humans in Babati district was unexpected because there were no control measures in place. These families could be exposed from other sources such as consumption of meat, blood and milk or assisting at calving and abortion on neighbours or relative houses. Close cohabitation in circumstances of poor hygiene, eating habits and livestock related activities (Chapter III) performed without protective measures (Niwael, 2001) could have resulted in high family seroprevalence in Ngorongoro district (Chapter V). Similar observations were reported in West African countries where eating habits and poor hygiene were thought to predispose people to infection (Unger and Munstermann 2004).

Families that were c-ELISA seropositive while their herds and flocks were c-ELISA seronegative presumably acquire the infection from other sources such as through drinking raw milk, assisting calving and handling aborted materials on neighbours farms. It was also observed that 25% of families were c-ELISA seronegative when their herds or flocks contained animals which were seropositive. One explanation could be the fact that in some families not all family members were bled. This may mask the true status of the disease at the family level. The practice of boiling milk observed in Chapter III in some households may be common in these households thus reducing the risk of human infection. Another possible explanation could be the recent introduction of infected animals into the herd or flock so there was not enough time for humans to acquire infection. Introduction of new animals into the herd or flock was positively associated with c-ELISA seropositivity (Chapter V).

In Chapter VI, the dynamics of brucellosis and its impact were explored. The incidence of brucellosis in pastoral households was 732/1,000 cases per animal-years at risk with an estimated survival probability of 0.836. The greatest proportion of new cases was seen in female domestic ruminants. The findings were similar to the cross-sectional study (Chapter V) where the greatest proportion of c-ELISA seropositive animals were female domestic ruminants. It was observed that households with higher seroprevalence at the initial sampling had a high incidence rate of seroconversion in the subsequent visit. This may be explained by the fact that high numbers of infected animals within a household may be an important risk factor in seroconversion (Lithg-Pereira, *et al.*, 2004). The presence of infected female animals in the household could be the source of infection within the herd or flock following parturition or abortion. Thus a high number of infected pregnant female animals in unvaccinated herds or flocks may increase the risk of transmitting *Brucella* infection following parturition or abortion (Radostits, *et al.*, 2000). Interestingly, the disease had a seasonal pattern with the highest incidence rate observed during the rainy season (wet season). This coincided with the parturition period and therefore, rain may exacerbate the transmission of brucellosis. Knowledge about the disease seasonal pattern may be useful in formulating appropriate control strategy and hygienic management for brucellosis in herds and flocks.

Some animals showed positive to negative seroconversion during the follow up periods. Animals which exhibited positive to negative seroconversion were categorised into two groups based on OD values. Animals with OD titres far from the cut-off OD value used in the study should be considered as "infected" and culled from the herd or flock. Absence of antibodies in the subsequent tests may not exclude infection as other animals remained

carriers (Bishop, *et al.*, 1994; Radostits, *et al.*, (2000). The second group of animals was those with OD values close to the cut-off OD value used in the c-ELISA test. These animals were probably false positives, and these have posed problems in the eradication programmes in several countries (Macmillan, 1990; Weynants, *et al.*, 1996; Pouillot, *et al.*, 1997). Culling such animals may be expensive and uneconomical thus further tests should be carried out to discriminate other possible cross-reacting organisms. The development of brucellin skin test has been a useful tool to discriminate false positive serological reactions (Pouillot, *et al.*, 1997; Bercovich, 2000).

An association between c-ELISA seropositivity and abortion and retained placenta observed in this study (Chapter V) indicated that brucellosis may cause economic loss through loss of calves and costs involved in the treatment of retained placenta. In this study it was observed that one case of retained placenta costs an average of US $2.4 when using antibiotics for treatment.

A statistical association was observed between calves and dams' seropositivity where calves born from seropositive dams were 27 times more likely to be seropositive compared to calves born from seronegative dams. Of the 17 calves that were seropositive, 82% were born from seropositive dams. Twelve percent of seropositive calves were born from seronegative dams. These calves were probably infected from grazing on contaminated pastures or suckling other cows. The small proportion of calves showed positive to negative seroconversion and some exhibited an undulating pattern in serology. Therefore, under such circumstances, it may be difficult to ascertain if calves have eliminated infection or if they remain carriers. In infected herds calves should be treated as suspicious

and if retained for breeding may continue to pose a risk to the herd. Bishop, *et al.*, (1994) suggested that suspicious calves should be removed from the breeding group. Where there is no compensation following testing, culling infected calves from the breeding stock is difficult. Where it is impractical, other alternative control measures could be developed aiming to reduce infection transmission. For example, in infected pastoral herds all positive male calves may be castrated and females kept up to adulthood and culled before breeding. In addition, colostral antibodies my influence this results as many calves' antibodies may decline to undetectable levels and become serologically negative even though a latent infection may exist in small proportions (Radostits, *et al.*, 2000). Colostral antibodies interfere with vaccination and screening testing thus advisable to conduct such activities after at least six months (Radostits, *et al.*, 2000).

There was no statistical association between heart girth measurements of calves suckled from positive and negative dams. Lack of a difference was probably attributable to several factors such as failure to determine the amount of milk each calf was getting and the practice of owners allowing calves to suckle from other cows. Alternatively, brucellosis may not have significant effects on milk production in the Tanzania Short Horn Zebu (TSHZ).

Although the longitudinal study generated some useful information on the dynamics of brucellosis in pastoral animals, the study had several limitations. Loss to follow up was a major problem, especially during the dry season which resulted in the need to exclude some of the households at the final analysis. Movement of animals due to searching for pastures and water during the dry season was inevitable in pastoral areas especially with

large herds and flocks. Longitudinal studies in pastoral livestock systems may therefore require consideration of livestock movements. Some of the practices of pastoral livestock keepers may hinder or interfere with data collection if not identified and addressed at an early stage with household members. Some taboos and human instincts such as valuing certain animals in the herd more than others and not allowing them to be included in the study and being reluctant to reveal the actual number of animals they own may influence the results. It is important to understand life style, cultures and taboos that related to livestock in order to avoid misunderstanding and to maximise the useful outputs of similar studies.

Therefore, longitudinal studies in African livestock have to consider the type of farming system as such a study may be possible in the smallholder dairy and agro-pastoral farming systems but difficult in the pastoral farming system. The following approaches may be helpful in pastoral systems:

(a) Longitudinal herds being selected conveniently in areas where movement of livestock is limited and the herds can be traced to grazing areas.

(b) If possible the study being conducted during rain season (A period of six months for Tanzania) before herds begin to move in search of pastures and water.

(c) Compliance has to be observed and the herd owner must be aware of all activities and their importance.

(d) Avoid herds from remote areas which may be inaccessible during rain season and render transport of samples that require immediate freezing difficult.

(e) Temporary crushes can be built in each household to facilitate sampling.

In Chapter VII isolation of *Brucella* organisms was attempted, as was validation of the RBPT as a field test in Tanzania. Isolation of *Brucella melitensis* type-1 from goats' milk has an important role to play in the epidemiology of human and cattle brucellosis in pastoral communities. Humans in the pastoral communities may be at risk of contracting *B. melitensis* infection by the consumption of raw milk from goats. During the PRA survey (Chapter III) it was revealed that milk was still consumed raw especially as soured milk. Keeping cattle and small ruminants in the same group with shared resources may predispose cattle to *B. melitensis* infection and pose risk to milk consumers and livestock keepers. *Brucella melitensis* infection in cattle has been reported in several countries of the Middle East (Refai, 2002). Lack of isolation of *B. abortus* in the current study may not rule out the possibility of the infection in cattle as previous studies had isolated the organism in cattle. Therefore, in infected areas, the use of S-19 and Rev-1 vaccines for cattle and small ruminants might be recommended.

Storage and transport of samples were also variable and not adequately and consistently controlled and could thus influence the culture positivity observed in the current study. Most of the field storage facilities did not either attain storage temperature at -20^0C or maintain that temperature due to power interruption while some of storage facilities are kerosene freezers. Transport of samples for long distances requires adequate cold facilities. Therefore, to have adequate storage in the field and during transport in field settings, liquid nitrogen containers will be recommended for future studies.

The results of the field RBPT validation in Chapter VII indicated that its agreement with c-ELISA was improved when the c-ELISA cut-off was adjusted from 60 to 70, but even so

the results were not as expected and the high occurrence of false negatives indicated that the RBPT antigen was either genuinely insensitive, and or other external factors made it insensitive. Also the performance of LRBPT was not satisfactory. An improvement of the test technique and the antigen are urgently required as this antigen is currently being used for screening animals and humans for brucellosis in Tanzania. The performance of RBPT in pastoral and agro-pastoral animals needs further improvement, and in areas with low seroprevalence two screening serological tests may be required to increase the likelihood of detecting positive reactors in the absence of a gold standard. Additionally, an alternative test can be used such as FPA which has shown to be useful in the field (Nielsen, *et al.*, 2002).

8.3 Recommendations

The following recommendations were the outputs of this study:

8.3.1 Technical support

Brucellosis seroprevalence was observed in livestock, humans and wildlife during this study. A mutual collaboration may be required so as to develop an appropriate serodiagnostic technique that will be applicable to all species. Veterinary Investigation Centres (VIC), health facility laboratories and wildlife laboratories need to be equipped with diagnostic kits for surveillance and routine diagnosis in hospitals. Indeed brucellosis screening may be included in the differential diagnosis of malaria and typhoid fever in humans in endemic areas since diagnosis based on clinical grounds has proved to be difficult.

8.3.2 Husbandry systems

Based on this study the seroprevalence and risk factors varied according to farming systems. Therefore, any intervention should consider the farming system in question. In the smallholder dairy and agro-pastoral systems the seroprevalence was relatively low. Following continued intensification of smallholder dairy in urban and periurban areas the level of brucellosis should be kept lower through active surveillance using either the Milk Ring Test (MRT) or serological tests and culling seropositive individuals to clear the remaining foci of infection and prevent introduction of infection into seronegative herds and flocks.

In the pastoral sector where seroprevalence was high, intervention may be difficult due to uncontrolled movement and transfer of animals. Test and culling in these herds may well be impractical. Thus, a combination of methods may be used such as vaccination against brucellosis using S-19 vaccine in cattle and Rev-1 vaccine in small ruminants that protect uninfected animals and allow them to remain in the contaminated environment, thus enabling infected animals to be disposed of gradually. This is important as compensation is not in place and is expensive. In this situation, isolation of animals at parturition and practicing good hygiene would be advisable.

8.3.3 Public health education

Effective education and publicity campaigns are necessary as part of control programmes and all accessible means of public information should be utilised.

To achieve this, mutual collaboration between veterinary departments, medical departments and local authorities is required. Village meetings and leaflets may be useful to encompass the major zoonoses highlighted in Chapter III. The message should include animals associated with the zoonosis, clinical signs in animals, means of transmission to humans, clinical signs in humans and possible ways to prevent disease occurring. The use of locally available protective materials such as plastic bags should be strongly encouraged if gloves are not available instead of using bare hands while assisting calving or handling aborted materials.

8.3.4 Economic evaluation

Control of brucellosis along with other infectious diseases prevailing in Tanzania may attract little attention. However, the disease burden in humans may justify its control. The seroprevalence of 8% in the pastoral and agro-pastoral communities indicated that the disease was prevalent in humans. The disability caused by the disease with expenses incurred during seeking medical services are enormous (Kunda *et al.*, 2004) and justify its control.

8.4 Future work

- The use of RBPT as a field and laboratory tests in Tanzania needs further improvement alternatively adopt another screening test such as fluorescence polarisation assay (FPA).

- Develop cost-effective and appropriate novel models for the control of brucellosis in pastoral communities in Tanzania which includes continuous education campaigns (proper disposal of aborted materials and placenta, general hygiene during calving and impact of the disease in livestock and humans), vaccination of cattle and small ruminants to protect uninfected animals while gradually culling infected animals and maintain active surveillance in high risk areas.
- Further studies to establish the *Brucella* species present in Tanzania may be imperative.
- The interaction between livestock and wildlife in maintaining the disease in the two populations require further epidemiological evaluation especially in Ngorongoro area where domestic ruminants and wildlife animals share all resources together.

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APPENDIX I

[The language used is Kiswahili-National Language in Tanzania]

(Hiki kijarida kimeandaliwa baada ya majadiliano bayana ya awali na kutolewa kama elimu ya afya kwa jamii zilizotembelewa wakati wa dodoso binafsi)

MAGONJWA HATARI AAMBUKIZWAYO BINADAMU KUTOKA KWA WANYAMA.

Karibu asilimia hamsini ya magonjwa yote yanayomwathiri binadamu yanatoka kwa wanyama (zoonoses). Magonjwa yanayomwathiri binadamu kutoka kwa wanyama yanaweza kuzuiwa kwa njia mbili kuu. Njia ya kwanza ni kuuzuia ugonjwa usimpate mnyama na ya pili ni kuuzuia ugonjwa husika usimpate binadamu.

Binadamu kuutambua ugonjwa ndiyo mwanzo wa kuuzuia/kuutokomeza ugonjwa huo. Baadhi ya magonjwa muhimu yawezayo kuambukizwa kwa binadamu kutoka kwa mifugo hapa Tanzania ni kichaa cha mbwa (rabies), kimeta (anthrax), kifua kikuu (Tb), brusela (brucellosis) na haidatidi (minyoo ya mbwa).

KUMTAMBUA MNYAMA MWENYE UGONJWA UNAOWEZA KUAMBUKIZWA KWA BINADAMU, JINSI UENEZWAVYO, DALILI KWA MNYAMA NA BINADAMU NA JINSI YA KUUZUIA.

1. KICHAA CHA MBWA

KUMTAMBUA MNYAMA

- Mbwa huwa na tabia isiyo ya kwaida (ukichaa)
- Hupenda kuuma kila kitu
- Hutoa mate hovyo
- Hukimbiakimbia hovyo

UENEZWAVYO

Kichaa cha mbwa huenezwa na mbwa mwenye kichaa anapomwuma binadamu

DALILI KWA BINADAMU NA JAMBO MUHIMU LA KUFANYA

Dalili kwa binadamu huanza kuonekana kuanzia wiki mbili na zaidi, nazo ni:-

- Kuumwa kichwa
- Kuchanganyikiwa
- Kupoteza fahamu
- Kifo

Mambo muhimu ya kufanya:-

- Sehemu mbwa alipouma paoshwe kwa maji ya uvuguvugu yaliyotiwa chumvi na sabuni huku mhudumu akitumia pamba au kitambaa kidogo. Mhudumu awe akikamua damu itoke huku akiosha. Hii husaidia kutoa mate na vijidudu vilivyo ndani ya kidonda.

Maji yenye chumvi, sabuni na kitambaa au pamba vitumike kuosha kidonda mara tu mtu anapoumwa na mbwa mwenye kuhisiwa kuwa na kichaa

- Mara mtu anapoumwa na mbwa asiyefahamika tabia yake/mwenye kichaa awahi hospitali haraka iwezekanavyo ili kupata chanjo.
- Ikiwezekana mbwa huyo afungiwe kwa siku 14 na ikiwa hajaonyesha tabia ya ukichaa inawezekana hana kichaa.

JINSI YA KUZUIA

- Hakikisha mbwa wako anapata chanjo kila mwaka
- Hamasisha wengine wachanje mbwa wao
- Ni muhimu mbwa wafungiwe

Kufunga mbwa hupunguza uwezekano wa maambukizi ya kichaa

Endapo utaona tabia ya mbwa wako imebadilika (kama zilizotajwa hapo juu) inabidi mbwa huyo auawe.

2. KIFUA KIKUU (Tb)

KUMTAMBUA MNYAMA

- Mnyama hudhoofu kwa muda mrefu wakati anakula vizuri na kutokua na homa
- Wakati mwingine aweza kukohoa

Ukweli ni kwamba ni vigumu kumtambua mnyama mwenye kifua kikuu kwani magonjwa mengine huwa na dalili kama hizi. Ni vizuri kumwona mtaalamu wa mifugo kwa ushauri zaidi.

UENEZWAVYO

- Kula nyama yenye ugonjwa, na ambayo ni mbichi au haijapikwa vizuri
- Kunywa maziwa yasiyochemshwa kutoka kwa mnyama aliye na kifu kikuu
- Kunywa damu mbichi kutoka kwa mnyama mwenye kifua kikuu
- Kuvuta hewa yenye vimelea vya kifua kikuu kutoka kwa mnyama mwenye kifua kikuu

DALILI KWA BINADAMU

- Kuwa na uvimbe kwenye tezi za shingoni
- Kukohoa mfululizo kwa muda mrefu na kudhoofu

- Kuwa na homa za muda mrefu na kutokwa na jasho usiku

Ni vizuri kumwona daktari mapema ili kujua kama ni Tb au ni ugonjwa mwingine.

JINSI YA KUZUIA

- Jitahidi kutumia nyama iliyopimwa na daktari wa mifugo
- Hakikisha nyama inapikwa kabla ya kutumia
- Chemsha maziwa kabla ya kunywa hata kama ni ya mtindi
- Epuka kulala nyumba moja na mifugo
- Epuka kutumia damu mbichi
- Kama una wasiwasi na mifugo yako muone mtaalamu wa mifugo

3. KIMETA

KUMTAMBUA MNYAMA

- Ni ugonjwa unaotokea ghafla
- Mara nyingi mnyama hukutwa amekufa
- Mnyama akifa huvimba sana, damu isiyoganda hutoka sehemu za wazi kama mdomoni, puani, sehemu ya kinyesi na kizazi na masikioni

UENEZWAVYO

- Kugusa mnyama aliyekufa, damu au nyama yake na hata ngozi yake
- Kula nyama au kunywa damu kutoka kwa mnyama mwenye kimeta
- Kuvuta vimelea vya ugonjwa kutoka kwa mnyama aliyeathirika au katika mazingira yaliyochafuliwa na hivyo vimelea

DALILI KWA BINADAMU

KWA KUGUSA

- Sehemu hasa za mikono hutokea vidonda. Vidonda vyaweza pia kutokea sehemu nyingine kama vimelea vimegusa sehemu hizo

KWA KULA

- Kuumwa tumbo na kuharisha ndiyo dalili muhimu

KWA KUVUTA VIMELEA VYA KIMETA

- Hii ndiyo njia hatari kuliko zote kwani mapafu huathirika na kuleta kifo (Kumbuka barua zilizowekewa vimelea huko Marekani ili watu wanapofungua wavute hivyo vimelea na kuleta maafa-silaha za kibaolojia)

JINSI YA KUZUIA

KWA MNYAMA

- Wasiliana na mtaalamu wa mifugo ili wanyama wako wapate chanjo ya kimeta

KWA BINADAMU

- Usile nyama au kunywa damu ya mnyama aliyekufa
- Iwapo utaona mnyama amekufa ghafla fikiria kwanza kimeta na chukua tahadhari ya kutomgusa
- Chunguza kwa makini dalili zake na muite mtaalamu ili afanye uchunguzi zaidi
- Mtu au mnyama mwingine asikaribie eneo alipofia huyo mnyama hadi mtaalamu atakaposema vinginevyo

IWAPO MTAALAMU HAYUPO FANYA YAFUATAYO:-

- Chimba shimo lenye urefu wa mita mbili
- Vaa gloves au mifuko ya plastiki mikononi
- Vaa kitambaa kufunika mdomo na pua ili usivute vimelea vya kimeta
- Chukua mzoga na udongo wa juu uliozunguka eneo alilofia uvifukie pamoja
- Fukia kwa udongo vizuri ili fisi na mbwa wasifukue

TAHADHARI! Iwapo kuna watu waligusa huo mzoga wanapaswa kwenda hospitali ili kupata dawa za kujikinga na uwezakano wa maambukizi

4 BRUSELA (Brucellosis)

KUMTAMBUA MNYAMA

- Ni vigumu kumtambua mnyama mwenye brusela
- Dalili inayoashiria brusela ni utupaji wa mimba kubwa hovyo bila ya mnyama kuonyesha kuwa na homa
- Kondo la nyuma hugoma kutoka baada ya kuzaa au kutupa mimba
- Kama dume la ngombe lina ugonjwa sehemu ya magoti na korodani huvimba

KUMBUKA KUWA: Hata magonjwa mengine yaweza kusababisha ngombe kutupa mimba hivyo ni vema mtaalamu wa mifugo akataarifiwa ili kupata ushauri zaidi

UENEZWAVYO

- Kunywa maziwa yasiyochemshwa kutoka kwa mnyama mwenye brusela
- Kumhudumia mnyama anapozaa/aliyezaa kwa kumshika mtoto wake, majimaji ya uzazi na kondo lake bila kinga kwenye mikono. Wadudu wa brusela wana uwezo wa kupenya kwenye vidonda na sehemu laini za mwili wa binadamu

Chukua tahadhari unapomsaidia mnyama akiwa anazaa

DALILI KWA BINADAMU

- Kuumwa na kicha
- Kuumwa na mgongo
- Homa za mara kwa mara
- Kutokwa na jasho usiku
- Kuumwa na viungo
- Mwili huchoka
- Mgongo kuuma

JINSI YA KUZUIA

- Wanyama wapate chanjo ya brusela
- Maziwa yachemshwe kabla ya kunywa au kutayarisha mtindi
- Epuka kunywa damu mbichi

Maziwa ni bora kwa afya yako ikiwa yatachemshwa kabla ya kunywa na hivyo kuzuia maambukizi ya brusela na Tb.

- Unapomsaidia mnyama katika kuzaa au aliyetupa mimba
    1. Vaa gloves au mfuko wa plastiki mikononi kabla ya kumsaidia
    2. Kama mnyama ametupa mimba hakikisha mtoto, kondo na uchafu wote vimefukiwa. Asipewe mbwa kwani ugonjwa waweza kumwathiri mbwa au kusambaza zaidi

Kama kuna mtu mwenye dalili zilizoorodheshwa hapo juu apelekwe hospitali mapema kwa uchunguzi zaidi.

5. HAIDATIDI (Hydatidosis)

Ni ugonjwa unaosababishwa na minyoo wadogo sana kutoka kwa mbwa. Huathiri binadamu na mifugo kama kondoo, mbuzi, ngo'mbe na punda.

UENEZWAVYO

- Mayai ya minyoo hutoka kwenye kinyesi cha mbwa na yanapoliwa kwenye majani au chakula chochote cha mifugo husababisha maambukizo kwa mifugo.
- Binadamu hupata kwa njia ya kula mayai ya minyoo hawa kwa bahati mbaya baada ya kushika mbwa au kinyesi chake au sehemu iliyokuwa na kinyesi cha mbwa na hatimaye kula kitu bila kunawa mikono.

Nawa mikono mara umhudumiapo mbwa, au hakikisha mbwa wako anapewa dawa za minyoo.

KUMTAMBUA MNYAMA

- Ni vigumu kumtambua mnyama aliyeathirika na ugonjwa huu hasa kwa mifugo walao majani
- Mbwa anaweza kuonyesha dalili za kudhoofu kama atakuwa na idadi kubwa ya minyoo.

DALILI KWA BINADAMU

Ni vigumu kugundua dalili za ugonjwa wa haidatidi, lakini dalili hizi zinaweza kuashiria ugonjwa huu

- Mgonjwa huwa na tumbo kubwa na mwili hudhoofu sana
- Anaweza kupata matatizo ya kupumua kama mapafu yameathirika

- Huwa na maumivu ya tumbo

USHAURI: Mgonjwa apelekwe hospitali uonapo dalili hizi la hasha aweza kupoteza maisha

JINSI YA KUZUIA

- Mbwa wapewe dawa za minyoo kila baada ya miezi mine
- Mbwa wasiruhusiwe kwenda machinjioni au kupewa nyama mbichi hata amechnjwa nyumbani
- Mtaalamu anapokagua nyama, sehemu zisizofaa zitupwe shimoni ambapo mbwa au fisi hawafikii
- Usicheze na mbwa ambaye hajapewa dawa za minyoo
- Nawa mikono kila mara baada ya kumhudumia mbwa

Imetayarishwa na:-

Mradi wa brusela:

Idara ya Tiba na Afya ya Jamii Chuo Kikuu cha Sokoine (SUA) na Taasisi ya Taifa ya Utafiti wa magonjwa ya binadamu (NIMRI), Tanzania

Kwa kushirikiana na:-

Vyuo vikuu vya Glasgow, Edinburgh na Liverpool-UK.

Imefadhiliwa na Shirika la Kimataifa la Maendeleo (DFID) la Uingereza

APPENDIX I

[English version]

(This was developed after the PRA study as a public health education leaflet and given after household cross-sectional questionnaire)

DANGEROUS DISEASES TRANSMITTED FROM ANIMALS TO HUMANS

More than 50% of all diseases affecting humans are zoonotic in nature. These diseases can be prevented at animal level or at human level.

Ability of people identifying the disease is an important step towards controlling or eradicating it. Examples of zoonotic diseases occurring in Tanzania include rabies, anthrax, brucellosis, tuberculosis and hydatidosis.

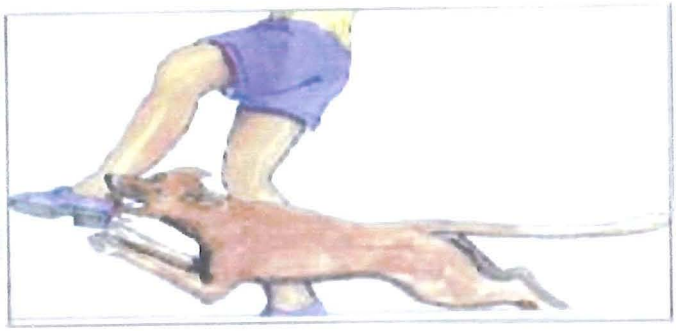
RECOGNITION OF ZOONOTIC DISEASE, HOW IS TRANSMITTED, CLINICAL SIGNS IN ANIMALS AND HUMANS AND HOW TO CONTROL THEM

1. RABIES

HOW TO RECOGNISE A RABID ANIMAL

- The dog will experience abnormal behaviour (madness)
- Biting everything ahead of it
- Excessive salivation
- Roaming around

TRANSMISSION



Rabies is transmitted when a rabid dog bites a human being

CLINICAL SIGNS IN HUMANS AND IMPORTANT STEPS TO TAKE

Clinical signs may be observed after two weeks or more and these include,

- Headache
- Confusion
- Loss of consciousness
- Death

Important things to do:-

- Use warm water mixed with salt and soap to wash the affected area. Cotton wool or a piece of cloth may be used by pressing the wound so as to allow more bleeding that helps to wash out the saliva and the virus.

Use warm water containing salt, soap with cotton wool or piece of cloth to wash the affected area immediately

- Anybody bitten by an unknown or rabid dog should report to hospital immediately for post vaccination
- If possible the dog should be confined for 14 days and if no abnormal behaviour developed then it is possible the dog is not rabid.

CONTROL MEASURES

- Vaccinate your dog annually
- Encourage others to vaccinate their dogs
- Dog confinement is important

Kufunga mbwa hupunguza uwezekano wa maambukizi ya kichaa

Confining dogs reduce transmission rates of rabies

If your dog has shown such behaviour listed above it needs to be killed.

2. TUBERCULOSIS (Tb)

HOW TO RECOGNISE AN ANIMAL WITH TB

- Progressive emaciation though the animal showed good appetite and with no fever
- Sometimes they cough

These signs are not pathognomonic as other diseases have similar signs. It is difficult to identify animals with Tb clinically. It is advisable to consult livestock experts.

TRANSMISSION

- Eating uncooked or undercooked infected meat
- Drinking raw milk from infected animals
- Drinking raw blood from infected animals
- Inhalation of contaminated air

CLINICAL SIGNS IN HUMANS

- Adenitis mainly around the neck
- Prolonged coughing
- Prolonged fever and night sweating

It is important to consult a doctor to confirm.

CONTROL MEASURES

- Consume meat inspected by meat inspectors
- Ensure meat is well cooked
- Boil milk before drinking or making it sour
- Do not cohabit with animals
- Cook blood before consumption
- If you suspect anything in your livestock consult a local veterinarian

3. ANTHRAX

HOW TO RECOGNISE AN ANIMAL WITH ANTHRAX

- It is a disease that occurs suddenly
- Frequently an animal is found dead
- A cadaver shows extended abdomen, oozing of blood from all orifices such as nose, mouth, vagina, anus and ears.

Transmission

- By contact with dead animal, its blood, meat or skin.
- Eating meat or drinking blood from infected animals.
- Inhaling the spores from infected animals or from the contaminated environment

CLINICAL SIGNS IN HUMANS

- **BY TOUCH**
- Skin lesion around the hands and other places came into contact with the pathogens.

- **BY EATING**
- Stomachache and diarrhoea are the main features.

BY INHALATION

- This is the most dangerous route where lungs are affected and results to death (Remember contaminated letters in the USA targeting to kill people following opening-Biological weapons)

CONTROL MEASURES:

IN ANIMALS

- Vaccinate your animals against anthrax by consulting livestock experts

IN HUMAN BEINGs

- Do not eat meat or drink blood from a dead animal.
- Think anthrax first when seen an animal died suddenly and do not touch it.
- Examine carefully the signs and call a livestock expert for further investigation. *Ensure neither people nor animal come close to the area unless otherwise stated by the livestock expert.*

DO THE FOLLOWING IF LIVESTOCK EXPERT IS ABSENT

- Make a pit of about 2 meters
- Wear gloves or any plastic materials in your hands
- Use a piece of cloth to cover the nose and mouth to prevent inhaling the pathogens
- Take the cadaver and the surrounding soils for burying
- Cover well to avoid dog and hyenas predation.
- **WARNING!** *Anybody who comes in contact with the cadaver will have to go to hospital for medication*

4 BRUCELLOSIS

HOW TO IDENTIFY AN ANIMAL WITH BRUCELLOSIS

- It is difficult to identify diseased animal clinically
- Major clinical signs are abortion at late stage without an animal showing signs of fever.
- Retained placenta following normal parturition or abortion
- Affected males will have hygroma and orchitis.

REMEMBER THAT: *Other diseases could cause abortion in animals therefore advice from livestock expert is necessary.*

TRANSMISSION

- Drinking raw milk from *Brucella* infected animals.
- Contact with infected animals during assisting parturition or abortion. *Brucella* organisms can penetrate the mucous membranes or abraded skins.

Take precautions when assisting parturition

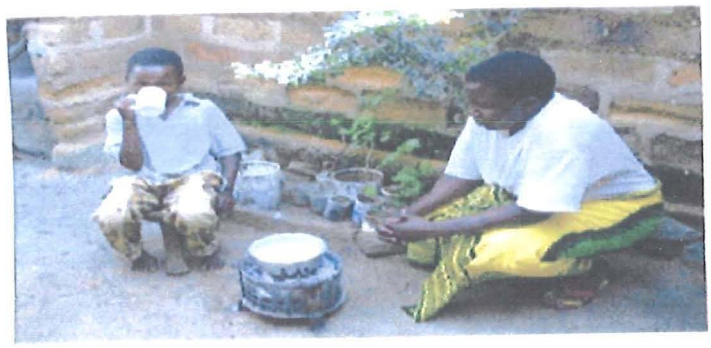
CLINICAL SIGNS IN HUMANS

- Headache
- Backache
- Recurrent fever
- Night sweating
- Joint pains
- Body malaise

CONTROL MEASURES

- Vaccinate animals against brucellosis

- Boil milk before drinking or preparing soured milk.
- Cook blood aimed for consumption



Boiled milk is good for your health and safe against brucellosis and tuberculosis

- When assisting parturition or abortion
    1. Wear gloves or plastic bags on your hands.
    2. Ensure aborted foetuses and other materials are buried. Do not give them to dogs as may infect dogs and possibly spread the disease further.

    Anybody with similar signs as mentioned above has to seek medical attention for further investigation.

5. HYDATIDOSIS

It is a disease caused by small worms of dogs. It affects both humans and livestock such as sheep, goats, cattle and donkeys.

TRANSMISSION

- Animals become infected when they consume pasture and feedstuffs contaminated with worm eggs
- Human beings are accidental hosts. They become infected by ingesting the worm eggs through contact with dogs, dog faeces or contaminated materials. This occurs by eating without washing hands after handling dogs.

Nawa mikono mara umhudumiapo mbwa, au hakikisha mbwa wako anapewa dawa za minyoo.

Wash your hands after handling dogs and ensure it is dewormed

HOW TO RECOGNISE AN ANIMAL WITH HYDATID WORMS

- It is difficult clinically to identify infected animals especially domestic ruminants.
- Dogs may show signs of emaciation when overwhelmed with worms.

CLINICAL SIGNS IN HUMANS

It is difficult based on clinical signs but the following may be suggestive:

- Extended abdomen with losing condition.
- May get breathing problems if lungs were affected.
- Stomachache

ADVICE: Anybody with such signs has to go to hospital otherwise may lead to death.

CONTROL MEASURES

- Deworm dogs after every four months.
- Restrict dogs in slaughtering places and do not give raw meat

- Following meat inspection the condemned parts are to be discarded appropriately where dogs and hyenas may not get access.
- Do not play with dogs that are not dewormed.
- Wash your hands once attending dogs.

PREPARED BY:-

BRUCELLOSIS PROJECT:

DEPARTMENT OF VETERINARY MEDICINE AND PUBLIC HEALTH SOKOINE UNIVERSITY (SUA) AND NATIONAL INSTITUTE FOR MEDICAL RESEARCH (NIMRI), Tanzania

IN COLLABORATION WITH:-

UNIVERSITIES OF GLASGOW, EDINBURGH AND LIVERPOOL-UK.

Sponsored by Department For International Development (DFID)-UK

APPENDIX II

EPIDEMIOLOGY OF HUMAN BRUCELLOSIS IN TANZANIA

DFID FUNDED RESEARCH PROJECT 2001

CROSS SECTIONAL QUESTIONNAIRE SURVEY ON BRUCELLOSIS (UNDULANT FEVER) IN HUMANS

1.0 BACKGROUND DATA

Study no..... Date of interview(dd/mm/yy).....

Head of household..... Sex..... Age (years).....

Interviewee's names.....

Sex..... . Age (years)..... Marital status.....

2.0 GENERAL INFORMATION

IMPORTANT: (Whenever the answer is volunteered, write V; for Yes and if prompted write P for Yes Always allow volunteer answers first by leaving the question open)

Are you aware of any human health problems associated with keeping animals?

Yes/No.....

If yes list at least five problems/diseases using English/Swahili/ Local names and list animals that may transmit the disease, clinical signs in animals, how each transmitted to humans and clinical signs in animals.

Disease	Animals involved	Signs in animals	Transmission to humans	Signs to people

3.0 FAMILY ACTIVITIES

Who is responsible for the following activities? (**Yes/No/NA.**)

If not around who else assists? (Write **Yes****)

	Milking		Herding	
	Cattle	Goats	Cattle	Goats and sheep
Husband				
Wife				
Son				
Daughter				
Attendant(employee)				
Relative				
Others (specify) eg				

Who slaughters at home? (**Yes/No**)

	Cattle	Goats	Sheep
Husband			
Wife			
Son			
Daughter			
Attendant			
Others (specify)			

What precautions do you undertake to control/prevent brucellosis in your family?

Boil milk	(V/P/X)
Handling of aborted foetus by wearing plastic bags	
Proper washing of hands with water immediately after assisting calving	
Cleaning the environment	
Boiling of water	
Cook meat	
Proper handling of aborted foetuses by wearing gloves	
Wash hands with soap immediately after assisting calving	
Do not allow anybody with cuts to assist calving or handle aborted foetus	
Others (specify) eg.	

5.0 CONSUMPTION HABITS OF ANIMAL PRODUCTS

Could you describe the source, preparation and consumption of milk in your family?

Source	Own cattle	Own goats	Shop	Neighbour	Relative	Others (specify)
Yes/No						

Milk destined for home consumption

Preparation	Fresh boiled	Fresh not boiled	Soured boiled	Soured not boiled
Yes/No				

If boiled, reasons for boiling milk. **(V/P/X)**

To kill pathogenic microorganisms eg.	
We have been told to boil milk from the hospital without reasons	
Now days people are advanced, so not accepting drinking milk which is not boiled	
Others (specify) eg.	

Reasons for not boiling milk.**(V/P/X)**

No enough time to boil milk	
Lack of firewood	
Calves could die if milk is boiled	
Butter fat decreases after boiling	
Taste and flavour becomes bad	
Boiled milk does not ferment properly	
Others (specify) eg.	

Who consumes milk in the family? **(Yes/No)**

Husband	
Wife	
Son	
Daughter	
Attendant (employee)	
Relative	
Others (specify) eg.	

Where did you get meat for your family in 2001?

Source	**Yes/No**
Shop	
Home slaughter	
Neighbour	
Auction market	
Wildlife	
Others (specify) eg.	

Do your family members consume blood? (**Yes/No**) .....

Where did you get blood for your family in 2001? (**V/P/X**)

Bled from live animal	
From slaughtered animal at home	
From neighbour	
From butcher	
From livestock auction market	
From relatives	
Others (specify) eg.	

How blood prepared (**V/P/X**)

Raw blood mixed with hot soup	
Raw blood mixed with milk	
Raw blood mixed with duodenal content, bile and offal chops like liver	
Fried/cooked	
Raw blood mixed with ruminal fluid (used as medicine)	
Others (specify) eg	

Who consumes blood in the family? **(Yes/No)**

Husband	
Wife	
Son	
Daughter	
Attendant (employee)	
Relative	
Others (specify) eg.	

If not consumed how is it disposed? **(V/P/X)**

Left to drain after slaughter	
Given raw to dogs	
Given cooked to dogs	
Cooked and prepare blood meal for livestock	
Others (specify) eg.	

APPENDIX III

INDIVIDUAL ANIMAL RECORD

Animal ID	*Sex*	*Age*	*Species*	*Parturitions*	*Last parturition*	*Parturition before last*	*Aborted*	*Retained placenta*	*Source*	*Current reproductive status*
Eg P110	F	2	Cattle	3	2002	2000	No	No	Home bred	Not pregnant
P111	M	4	Goat	NA	NA	NA	NA	NA	Brought	Castrated

APPENDIX IV

EPIDEMIOLOGY OF LIVESTOCK BRUCELLOSIS AND ITS IMPACT ON LIVESTOCK HEALTH AND PRODUCTION IN TANZANIA

DFID FUNDED RESEARCH PROJECT 2001

CROSS SECTIONAL QUESTIONNAIRE SURVEY ON BRUCELLOSIS IN LIVESTOCK

1.0 BACKGROUND DATA

Study no..... Date of interview(dd/mm/yy).....

Head of household..... Sex..... Age.....

Interviewees names..... Sex..... . Age.....

Marital status.....

Kitongoji..... Village..... Ward.....

Division..... ..... District.....Ten cell leader.....

2.0 GEOGRAPHIC INFORMATION

GPS coordinates.....S.....E Altitude(m).....

Distance to nearest neighbour (meters/km).....

Distance from village centre (meters/km).....

3.0 HERD MANAGEMENT

How many youngstock were born in the herd/flock in wet and dry seasons during the year 2001?

(Give exact figure or range)

Youngstock	Dry season	Wet season
Calves		
Kids		
Lambs		

How many youngstocks were born in the following areas in 2001? (give approximate figures and if no birth in 2001 write NA)

Place	Calving	Lambing	Kidding
At home in the house			
At home in the boma			
At home outside the house			
At home outside the boma			
Outside on the pastures nearby			
On the grazing grounds far from home			
Other places (specify) eg.			

IMPORTANT: ***(Whenever the answer is volunteered, write V for Yes; and if prompted write P for Yes and if No write X. Always allow volunteer answers first by leaving the question open)***

How did you manage to arrange parturition in this period in 2001? **(V/P/X/NA)**

Animals/control	Separate breeding males and castrate the rest	Prevent males from mating by using skin/plastic sheaths around the prepuce	Divide animals into groups of males and females	Others (specify) eg.
Cattle				
Goats				
Sheep				

Did any of your animals abort in 2001? **(Yes/No)**.....

If **yes** indicate how many and the stage of abortion:

Animals/stage	Early	Mid	Late	Unknown
Cattle				
Goats				
Sheep				

What methods of disposing aborted foetus did you use in 2001?

Where	(V/P/X)
Thrown raw to dogs	
Given to dogs after cooking	
Thrown into the bush	
Buried	
Burned	
Eaten by family members	
Others (specify)	

Have you had any cases of retained placenta in 2001? **(Yes/No).....**

If **Yes**; how many cases of retained placenta in (a) cattle.....(b) goats..... (c) sheep.....

Which method was used to dispose retained placenta in 2001.

Where	**(V/P/X)**
Thrown raw to dogs	
Given to dogs after cooking	
Buried	
Burned	
Thrown in the bush	
Just left where it falls	
Others (specify) eg.	

How was manure handled in 2001?

Where	**(V/P/X)**
Collected outside the boma/house	
Taken out to dry and returned as bedding	
Collect outside and take to field	
Collect and sell to people	
Collect and give to neighbours	
Use to plaster houses	
Use for biogas	
Use for burning	
Used to plaster pots and storage bins	
Others (specify) eg.	

Do you have female animals that have given birth before 2001 but that now failed to conceive? **(Yes/No)**.....

If **yes** how many:

Animal	Cattle	Goats	Sheep
Total			

Why do you think this problem happens? **(V/P/X)**

Reasons	Cattle	Goats	Sheep
Lack of breeding males in the herd			
Problems from previous parturition. eg.			
The animal resents being mounted			
Failure to detect heat on time			
Animals are too old			
Problem of getting male on time			
The animal do not show clear heat signs			
Failure to detect heat on time			

Lack of money to hire a male for service on time			
Male animal is tired of service			
Others (specify) eg.			

What did you do with such animals in 2001? **(V/P/X)**

	Cattle	Goats	Sheep
Just left in the herd			
Slaughtered at home			
Sold for slaughter			
Given as gift			
Given out as dowry			
Others(specify)			

5.0 LIVESTOCK MOVEMENT AND CONTACTS

Contact with other animals (**1**=often, **2**=occasionally, **3**=never)

HERDS	DRY SEASON		WET SEASON	
	Grazing areas	Watering points	Grazing areas	Watering points
Cattle from other herds				
Sheep/Goats from other herds				
Wild animals				

Where do you keep your animals at night? **(V/P/X)**

Place at night	Cattle	Goats	Sheep
In the house with family members			
In the house without family members			
Outside in the boma			
Others (specify) eg.			

Do your animals stay with other animals during the night? **(Yes/No)**.....

If **Yes,** why do you mix with other animals?

For security reasons	V/P/X
As relatives we put animals together	
Animals on transit stay for few days	
Others (specify) eg.	

Are the cattle herded with sheep and goats?

During dry season **(Yes/No)**..... During wet season **(Yes/No)**.....

Did you acquire any new livestock in 2001? **(Yes/No).....**

If **yes;** indicates the origin and numbers acquired

	Village	Total	Market	Total	District	Total
Cattle						
Goats						
Sheep						

How many livestock do you own? (Give exact figure or range)

Animals	Interviewees response		Direct observation	
	Females	Males	Females	Males
Cattle				
Goats				
Sheep				
Donkeys				
Pigs				
Calves	(Indicate regardless of sex)			
Kids				
Lambs				
Piglets				

5.0 WILDLIFE IN THE AREA

How frequently do you see the following wildlife species in the following areas? (1=often, 2=occasionally, 3=Never)

Species	In the grazing grounds	Direct observation
Dikdik		
Wildebeest		
Buffalo		
Zebra		
Elephant		
Impala		
Thomson Gazelle		
Giraffe		

6.0 Name of the interviewer.....