The epidemiology of human brucellosis in the context of zoonotic diseases in Tanzania

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Declaration

I hereby declare that the research described in this thesis is my own original composition, that the thesis is my own work and it has never been submitted for any other degree or professional qualification

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Dedication

This work is dedicated to all members of my family who missed me so dearly during the times of data collection, analysis and write-up. It is also dedicated to all those who assisted me in many different ways to achieve this academic level.

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Abstract

The aims of the study were to determine the seroprevalence and incidence of human brucellosis in Arusha and Manyara regions, risk factors for human brucellosis, health-seeking behavior and clinical features of human brucellosis cases and to evaluate different diagnostic tests for brucellosis. Other objectives included determination of the knowledge of medical practitioners relevant for diagnosis of zoonoses and estimation of the burden of disease caused by human brucellosis in Tanzania.

From cross-sectional studies, the *Brucella* seroprevalence in humans was 4.8% when determined by assays in the field and 6.4 % at the Sokoine University of Agriculture (SUA) based on the Rose Bengal Plate Test (RBPT). Based on the competitive ELISA (c-ELISA) test conducted at the Veterinary Laboratory Agencies (VLA) the seroprevalence of brucellosis was 7.7%. The majority of RBPT positive individuals were asymptomatic. Most of the c-ELISA positive individuals were above 15 years of age with the age group 16-30 years having the highest number of seropositive individuals. There was a significant association between the seroprevalence of brucellosis in humans and the seroprevalence of brucellosis in goats at the district level.

Prospective hospital studies indicated that the incidence of brucellosis was 11.2 cases/100,000 people per annum. Joint pain, headache, backache, fever and fatigue were the main clinical features described by the confirmed (c-ELISA positive) patients, but these were also most commonly reported by the c-ELISA negative patients initially suspected as having brucellosis. Patients with brucellosis delayed going to hospital with a median delay time of 90 days. Distance to the hospital, keeping animals and knowledge of brucellosis were significantly associated with patient delay to present to hospital. More cases of brucellosis were recorded in hospitals located in pastoral areas and brucellosis was more common among people engaging in business.

Brucellosis was associated with assisting an aborting animal. It was shown that the closer the distance between households, the higher the risk of brucellosis. People who were of Christian religion were found to have a higher risk of disease compared to other religions.

The sensitivity and specificity of the RBPT in the cross-sectional survey were 39.4% and 98.8% respectively, at the SUA laboratory 38.7% and 96.8% respectively and at the hospitals 44.3% and 89.5% respectively. The sensitivities and specificities of the diagnostic tests for brucellosis at the hospitals were also low. There was a poor agreement between the RBPT performed at SUA, the RBPT performed in cross-sectional survey and the tests performed at the hospitals.

Medical practitioners in rural hospitals had poorer knowledge of most zoonoses when compared to the practitioners in urban hospitals, including transmission of sleeping sickness, clinical presentations of anthrax and rabies in humans. In both areas practitioners had poor knowledge of echinococcosis transmission to humans, clinical features of echinococcosis in humans, and diagnosis of bovine tuberculosis in humans.

Brucellosis contributed to an estimated 3,644 -3,708 Disability Adjusted Life Years (DALY) burden in Tanzania based on data collected from hospitals while data from the community resulted in an estimated 92,080 – 121,550 DALY burden in Tanzania. The majority of cases continued to have brucellosis clinical features for a period of over two years, and out of these, five days spent as inpatients. Households used a mean total of US $ 90.65 (92, 826 TShs.) to care for a single case of brucellosis per year and each health provider used a mean total of US $ 858 (878,592 TShs.) per year to care for cases of brucellosis.

Brucellosis contributes to poverty and suffering particularly to the poor in the rural areas of Tanzania and yet it is neglected. There is a need for increased health education on risk factors for transmission of brucellosis to humans and the importance of going to hospital at an early stage of the disease. More efforts also need to be directed towards improving the diagnosis and treatment of brucellosis to reduce prolonged human suffering from brucellosis. This should include the adoption of standardized diagnostic and treatment protocols. Restructuring and updating of disease recording systems and diagnostic laboratories so that they diagnose and hence capture zoonoses such as brucellosis should be implemented. Efforts should be made to equip practitioners with adequate knowledge relevant for identification of zoonoses.

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Abbreviations and acronyms

AIDS	Acquired Immune Deficiency Syndrome
HIV	Human Immunodeficiency Virus
CDC	Centres for Disease Control and Prevention
c-ELISA	competitive Enzyme-Linked Immunosorbent Assay
RBPT	Rose Bengal Plate Agglutination Test
SAT	Serum Agglutination Test/Standard Agglutination Test
CFT	Complement Fixation Test
df	Degrees of Freedom
^{0}C	Degrees Celsius
FAO	Food and Agriculture Organization
WB	World Bank
WHO	World Health Organization
VLA	Veterinary Laboratories Agency
SUA	Sokoine University of Agriculture
TShs.	Tanzanian Shilling
US$	United States Dollar
UK	United Kingdom
NIMR	National Institute for Medical Research
Se	Sensitivity
Sp	Specificity
Ppv	Positive predictive value
Npv	Negative predictive value
HAT	Human African Trypanosomiasis
TB	Tuberculosis
GDP	Gross Domestic Product
GBD	Global Burden of Diseases
DALY	Disability Adjusted Life Year
YLL	Years of Life Lost due to premature death
YLD	Years Lived with Disability

2ME	2-Mecapto Ethanol
BVD	Bovine Viral Diarrhoea
NACP	National Aids Control Programmes
Ltd	Limited
Co	Company
USA	United States of America
NEDLIT	National Essential Drugs List for Tanzania
DFID	Department For International Development
CI	Confidence Interval
OR	Odds Ratio
YI	Youden's Index
ROC	Receiver Operating Characteristics
OD	Optical Density

1. General Introduction

1.1 Zoonoses

Zoonoses are diseases that can be transmitted between humans and vertebrate animals (WHO, 1959; Palmer *et al.*, 1998). In most cases, animals play an essential role in maintaining the infection in nature, and they contribute in varying degrees to the distribution and transmission of infection in human and animal populations (Woolhouse and Gowtage-Sequeria, 2005). Zoonoses account for 61% (n=868) of all human diseases and 75% of all emerging human pathogens (Taylor *et al.*, 2001). Zoonoses, however, are diseases where lack of uptake of the available control strategies is constrained by more than just access and affordability in developing countries (Schwabe, 1984). From a veterinary perspective, the most effective control strategy in animal diseases is to deal with the animal itself, while in human disease it is to deal with the patient. Zoonoses therefore, fall between the two sectors, which are already over-stretched and under-funded (Schwabe, 1984).

In sub-Saharan African countries many of the known zoonoses, which are poorly controlled in both livestock and human populations, endanger poor people's livelihoods by affecting their livestock and compromising their health and survival. Zoonoses also cause great economic losses to poor people particularly in the rural areas of sub-Saharan African countries (Perry *et al.*, 2001). The losses caused are both direct (morbidity, mortality to both humans and livestock, and fertility problems, milk yield and ability of an animal to work as a traction animal), and indirect, such as those due to the costs of ineffective control measures, limiting production opportunities, and influencing the choices made by livestock keepers and farmers (Perry *et al.*, 2001; Shaw, 2004).

Zoonoses such as bovine tuberculosis, brucellosis, anthrax, sleeping sickness, and rabies are still widespread in Africa (Meslin, 1992; Barrett, 2006). In Tanzania, African trypanosomiasis, plague, rabies, brucellosis, anthrax and hydatidosis have been documented as being among the most common zoonotic diseases (Kilonzo and Komba, 1993), however little quantitative data is available to substantiate this finding. Bovine tuberculosis, tetanus, taeniosis and trichinosis are also endemic in some parts of the country although epidemiological data on their incidence is scarce (Kilonzo and Komba, 1993). Multidisciplinary, inter-programmatic and cross-cultural efforts by the health, agricultural, environmental and other sectors of society at the national level have been suggested as ways of controlling and preventing zoonotic diseases (Meslin, 2005). Effective control of zoonoses also requires strong regional and international cooperation and prompt notification of disease occurrence domestically, regionally and globally (Meslin, 2005).

Rabies is mainly transmitted through a bite of a rabid animal which, in most parts of the world, is by a dog bite. An estimated 35,000 to 50,000 human deaths are caused by rabies each year (Knobel et al., 2005). More than 99% of all human rabies deaths occur in the developing world, despite the availability of effective and economical tools for prevention and control (Knobel et al., 2005). The low political commitment to rabies control leads to a lack of accurate data and underestimation of the true public health and economic impact of the disease in most of the developing world (WHO, 2004; Miranda, 2005). The incidence of rabies in Tanzania was estimated to be 100 times higher than officially recorded, with 1499 human deaths annually in comparison with the 10-20 human cases typically reported each year by central authorities (Cleaveland et al., 2002). Apart from human morbidity and mortality,

rabies also contributes significantly to livestock loss (Laurenson *et al.,* 1997; Knobel *et al.,* 2005).

Cystic echinococcosis (hydatidosis), caused by the larva stage of the tapeworm *Echinococcus granulosus* is highly prevalent in many developing countries, especially in poor communities (Eckert and Deplazes, 2004). It has been reported in West African as well as the East African countries. In all these areas, hydatidosis is prevalent in humans among the nomadic pastoralists (Magambo *et al.,* 2006). Due to the lack of well-documented data from many countries in Africa, the sub-Saharan picture of the current hydatidosis situation is incomplete (Magambo *et al.,* 2006).

Hydatidosis causes serious human suffering and considerable losses in agriculture and human productivity (Budke, 2006). The transmission of the disease is linked with general lack of knowledge on transmission factors and prevention measures among the population at risk, poor inspection of meat before human consumption, the abundance of stray dogs, and improper disposal of animal offal and home slaughtering exercises which are common practises in many rural areas of developing countries (Budke, 2006). The incidence of surgical cases of hydatidosis range from 0.1 to 45 cases per 100,000 people and the real prevalence ranges between 0.22% and 24% in endemic areas (Kachani, 2005). In Libya, Morocco and Tunisia the prevalence of surgical hydatidosis ranged from 1%-2% (Kachani, 2005). Intensive strategies for health education on the transmission routes of hydatidosis, improvement in abattoir conditions and management of the dog population have been cited as among the most important control strategies (Kachani, 2005).

Human anthrax is most common in enzootic areas in developing countries, among people who work with livestock, eat undercooked meat from infected animals, or work in establishments where wool, goatskins, and pelts are stored and processed (Turnbull, 2002). West Africa is the most affected area of the world (Hugh-Jones, 1999). Anthrax is also a significant problem in other parts of Africa, Central America, Spain, Greece, Turkey, Albania, Romania, central Asia, and the Middle East (Hugh-Jones, 1999). Between 1979 and 1985, in association with the civil war and the interruption of veterinary public health practices, Zimbabwe was the site of the largest outbreak of anthrax, with about 10,000 human cases, almost all of which were cutaneous infections (Davis and Elzer, 2002; Davies, 1985). In Tanzania, an epidemic of 239 human cases of anthrax was reported in Rukwa region in 1985 due mainly to consumption of meat from infected animals dying of the disease. Sporadic cases have also been reported in different parts of the country (Webber, 1985).

Zoonotic tuberculosis (TB) caused by *Mycobacterium bovis* is present in most developing countries, where surveillance and control activities are often inadequate or unavailable; therefore, many epidemiologic and public health aspects of infection remain largely unknown (Cosivi *et al.*, 1998). Although prevalence data on animal TB in developing countries are generally scarce, information on bovine TB occurrence and control measures exists in some countries (Thoen *et al.*, 2006). Of 55 African countries, 25 reported sporadic/low occurrence of bovine TB in 1999; six reported enzootic disease; two, Malawi and Mali, were described as having a high occurrence; four did not report the disease; and the remaining 18 countries did not have data (Thoen *et al.*, 2006).

Of all nations in Africa, only seven apply disease control measures as part of a test-and-slaughter policy and consider bovine TB a notifiable disease; the remaining 48 control the disease inadequately or leave it uncontrolled. Thus, approximately 85% of the cattle and 82% of the human population of Africa are in areas where bovine TB is either partially controlled or it is uncontrolled (Cosivi *et al.*, 1998). The study conducted by Kazwala *et al.*, (2001) in Northern and Southern zones of Tanzania revealed that 70.5% (n=149) of the *Mycobacterial* isolates of human cervical adenitis recovered from all forms of tuberculosis were identified as *M. tuberculosis*, 16.0% were identified as *M. bovis,* and 13.6% were other *Mycobacterial* species. There was a significantly higher isolation rate (P < 0.05) of *M. bovis* among strains recovered from extra-pulmonary (26.8%) than pulmonary tuberculosis samples (4.3%) (Kazwala *et al.*, 2001).

The prevalence of Human African Trypanosomiasis (HAT) in Africa has been estimated by the WHO to be 300,000 cases over the last ten years, an average of 30,000 new cases annually in the whole African continent (WHO, 2003). The figures, however, are thought to be an underestimation as a result of a large number of people (60 million) living in risk areas and having limited access to diagnostic and health care facilities (Cattand *et al.*, 2001). In Uganda it was estimated that for every case of a *Trypanosoma brucei rhodesiense* death in hospital, about 12 additional cases occur in the community undetected (Odiit *et al.*, 2005). In Tanzania the disease is prevalent in the western, north, and northwestern parts, the southern highlands and southern regions, where over 6000 cases have been reported since 1979. Control strategies against sleeping sickness in Tanzania include chemical control of vectors, treatment of patients with trypanocides and avoidance of human tsetse contact (Kilonzo and Komba, 1993).

Plague caused by *Yersinia Pestis* is mostly endemic in central, northern and north-eastern Tanzania. A total of 8161 cases with 1885 deaths have been recorded since 1890. The disease is currently prevalent in Lushoto district where outbreaks have been experienced since 1980, and in Singida district where it has been endemic since 1918 (Kilonzo and Komba, 1993). Integrated control measures such as rodent control were possibly responsible for the 1989 decline of outbreaks in the area. Financial constraints which led to deterioration of control activities from July 1989 probably accounted for the severe outbreaks in 1990/91 which then spread to other parts of the country (Kilonzo and Komba, 1993).

1.2   Farming systems in Tanzania

Livestock contribute to the livelihoods of at least 70% of the world's rural poor (Miranda, 2005). Communities, especially in rural areas, have traditionally depended on animals for food, transport and farm work. This dependence increased with intensification in livestock production and the increase in the transport of livestock and their products domestically and across the globe (Miranda, 2005). In Tanzania, about 90% of the population live in rural and peri-urban areas, owning more than 80% of the total livestock population. Around 60 million hectares of rangeland is suitable for livestock grazing on which approximately 106,000 households practise pastoralism and approximately 268, 000 are engaged in agropastoralism (Ministry of Agriculture and Cooperatives, 1995b). Tanzania has a large livestock population comprising 13 million cattle, 3.7 million sheep, 6.4 million goats, 275,000 pigs and 22 million chickens. The livestock sector, which forms an important part of the agricultural sector, contributes more than 18% of the Tanzanian Gross Domestic Product (GDP) (Ministry of Agriculture and Cooperatives, 1995a).

The pastoralist economy is based on migration and livestock rearing. Although humans and livestock migrate along conventional routes, movements can vary with climatic and environmental conditions, for example in one dry season pastoralists may visit certain wells and pastures and during the next dry season other wells and pasture (Imperato, 1974). Pastoralists derive most of their sustenance and livelihood directly from livestock. Agro-pastoralists systems combine crop and animal production, using outputs from one to feed into the other, e.g. manure for crops and fodder for livestock. This is the most common mixed farming system in Tanzania (Imperato, 1974; Ole Kuney, 1994).

Pastoralists aim to keep their livestock herds as large as possible to secure adequate milk and meat supplies and to gain influence and respect within their communities (Stark and Protz, 1973). Studies, however, have shown the incidence of brucellosis to be highest in the pastoral system where it decreases with herd size and as land holding decreases (McDermot and Arimi, 2002).

1.3 Brucellosis

Brucellosis is an acute or chronically contagious disease of animals and humans, caused by species of the genus *Brucella* (Corbel, 1989a). The genus can be divided into six species, namely *Brucella abortus*, *B. melitensis*, *B. canis*, *B. ovis*, *B. suis* and *B. neotomae*, based on pathogenesis and host predilection. In 1994, the isolation of *Brucellae* was reported in marine mammals and was named "*B. maris*" (Aleixo *et al.*, 1999). Although recent bacterial nomenclature has re-classified these species as biovars within a single species *Brucella melitensis* (Verger *et al.*, 1998), this study will continue to refer to the species as given above, which is still in common usage.

Four of the species (*Brucella abortus, B. melitensis, B. canis* and *B. suis*) can infect man by direct or indirect contact with infected animals (Corbel and Brinley-Morgan, 1984). The primary and secondary host species for the six *Brucella* species are shown in Table 1.1.

Although brucellosis can be found worldwide, it is more common in areas of the Mediterranean basin, South and Central America, Eastern Europe, Asia, Africa, the Caribbean and the Middle East. In most developing countries brucellosis is a zoonosis of both public health and economic significance because animal diseases have not been brought under control (Perry *et al.*, 2002). As a consequence, there has been an increase in the number of human cases of brucellosis in developing countries (Refai, 2002) because the occurrence of the disease in humans is largely dependent on the animal reservoir (McDermot and Arimi, 2002). Brucellosis has been eradicated in many developed countries including the UK since 1980-81 through vaccination campaigns combined with test and slaughter policies in livestock (Emslie and Nel, 2002). Even in these countries, however, human infections still occur as a result of imported cases from endemic areas (WHO, 1997).

Table 1. 1: *Brucella* species and their hosts

Species	Primary host	Other hosts infected
B. abortus	Cattle	Camels, deer, sheep, Goats, pigs, dogs, humans and deer
B. melitensis	Goats and sheep	Cattle, humans
B. suis	Swine, reindeer, small rodents and humans	
B. canis	Dogs	Humans
B. ovis	Sheep	
B. neotomae	Desert wood rats	
B. maris	Marine mammals	Humans

1.4   History of brucellosis

History tells that Hippocrates first described a disease syndrome similar to brucellosis around 450 BC. An early description of brucellosis was made by Martson in 1859 (Vassalo, 1992). He wrote of illness, including his own, which differed from typhoid fever. The aetiology of the disease in man was later classified when Captain Bruce, in 1887, isolated *B. melitensis* from the spleen of a dead soldier in Malta and from the blood of a living patient, and named it *Micrococcus melitensis* (Nicoletti, 2002). In animals, *Brucella* was isolated by a Danish Professor by the name of Bernard Bang in 1895 from the membranes and uterus of cattle following abortion. He later confirmed the aetiology of the disease when he produced it in healthy pregnant heifers using pure cultures of the bacillus. He named it 'Abortus bacillus of Bang' (Nicoletti, 2002).

In 1905, a scientist named Harrocks was able to demonstrate the *Brucella* organism in milk, and this discovery was later followed by strict rules on consumption of pasteurized milk resulting in a dramatic decrease in the incidence of the disease in the Mediterranean area (Nicoletti, 2002). *B. suis* was discovered by Traum in the United States in 1914 from aborted cows. The first case of *B. abortus* was described by Keefer in the United States in 1924. The three species of *Brucella, B. abortus, B. melitensis* and *B. suis* were clearly distinguished by Huddleson in 1929. *B. ovis* was isolated from sheep in Australia in 1953, *B. neotomae* from desert wood rats in United States in 1957, and *B. canis* from dogs in the United States in 1968 (Nicoletti, 2002). More recently, a species of *Brucella* was isolated in 1994 from marine mammals and was named "*B. maris*" (Aleixo *et al.*, 1999).

1.5   *Brucella* organisms

Brucellae are intracellular parasites that infect a wide variety of domesticated and free-living animals. *Brucellae* grow intracellularly and localize in the reticulo-endothelial system in the genital tract and the mammary glands of their respective primary hosts (Hurvell *et al.*, 1971; Verger *et al.*, 1998). Growth of *Brucellae* occurs aerobically, often enhanced by CO_2, however no growth occurs under strict anaerobic conditions (Verger and Grayon 1977).

Brucellae are small round or oval cocco-bacilli, they are arranged singly, sometimes in pairs, short chains or small clusters, they do not produce capsules, spores or flagella. *Brucellae* are gram negative, do not usually show bipolar staining, are not acid-fast but may resist decolorization by weak solutions of acids or alkalis (Verger *et al.*, 1998).

1.6   Diagnosis of brucellosis

Clinical features and laboratory investigation form the basis for the diagnosis of brucellosis in humans (FAO/WHO, 1985). Although the definitive diagnosis of brucellosis is by culture and isolation of the causative organisms, the procedure is challenging in that it requires special media, takes several weeks of incubation and has low sensitivity (Gotuzzo et al., 1986; Araj et al., 1990). The laboratory diagnosis of brucellosis therefore often depends on serologic tests. These include the Serum Agglutination Tests (SAT) (Corbel and Brinley-Morgan, 1984), the Complement Fixation Test (CFT) (Alton et al., 1983), the Fluoroscent Polarization Assay (FPA) (Nielsen et al., 2000; Lucero et al., 2003) and the Enzyme-Linked immunosorbent Assays (ELISA) tests (Lucero et al., 1999). Other tests include the radioimmunoassay (Parratt et al., 1977), the indirect immunofluorescence assay (Colmenero et al., 1989), and the 2-mercaptoethanol test (2ME) (Reddin et al., 1965).

1.6.1   The SAT

The SAT is the agglutination test used in sero-prevalence studies, and has been used in sero-diagnosis of bovine brucellosis in Tanzania (Mahlau and Hammond 1962; Starck and Protz, 1973). During the acute phase of the illness, repeated serum samples should be collected to demonstrate a rising (greater than four –fold) titre of *Brucellae* agglutination tests. High titres are usually encountered by the second or third week of illness, with titres of 1/80 or higher (Corbel, 1972).

In the sub-acute or chronic phase of brucellosis, the standard agglutination test may be particularly difficult to interpret or may be negative and other tests need to be done to confirm the results. This is because the serum agglutination test depends

very much on the presence of IgM immunoglobulin that could be low or absent in chronic and sub-acute states. This also explains why the SAT is negative during the incubation period and following abortion (Mittal and Tizard, 1983).

Other difficulties that can be encountered with the use of the SAT include cross-reactions with bacteria related to the genus *Brucella,* and positive results in exposed populations and in patients after they are cured (Diaz *et al.,* 1982). This phenomenon is probably due to the presence of common antigenic determinants in the lipopolysaccharide fractions of *Brucella abortus* 99 and *Yersinia enterocollitica* 09 (Zheludkov, 1982). Strains of *B. abortus* also share antigenic properties with strains of *E. coli* O157 (Caroff *et al.,* 1984).

In animals, serological tests cannot differentiate between cattle infected with *Brucella* and cattle infected with microorganisms that serologically cross-react with *Brucella* antigen, which is likely to affect test specificity. Hence, these cattle and cattle with 'natural' antibodies jeopardize the *Brucella*-free status of a herd. Infected cattle with serologically inconclusive test results, or which elude detection, are also a hazard to *Brucella*-free herds (Bercovich, 1998). The most common agglutination test for brucellosis in current usage is the RBPT (Davies, 1971) see sections 1.6.2 and 2.4.5.1.

1.6.2 The RBPT

The RBPT is an agglutination test that provides results in 2-4 minutes and is useful as a first screening test (Davies, 1971). The RBPT was designed originally for screening in veterinary medicine, but is now often used for the diagnosis of human

brucellosis (Morgan *et al.*, 1969; Oomen and Waghela, 1974). Its ease and speed of use, as well as its low cost, have made it very popular in hospitals and field conditions for the diagnosis of brucellosis (Colmenero *et al.,* 1989). The protocol for the RBPT performed in this study is described in section 2.4.5.1 and the performance of the RBPT in different places has been discussed in chapter 7 of this thesis.

1.6.3 The c-ELISA test

The c-ELISA tests are based on primary binding assays, mainly to improve test sensitivity and specificity. The c-ELISA test for the detection of serum antibodies to the organisms of the genus *Brucella* has been shown to be a suitable test for human brucellosis (Lucero *et al.,* 1999) see section 2.4.5.2. The c-ELISA test uses a monoclonal antibody (mAb) specific for a common and repeating epitope on the polysaccharide portion of the smooth lipopolysaccharide molecule of *Brucella* (S-LPS) to compete with antibody in the sample. This results in an assay with higher specificity than other assays because it frequently eliminates cross-reactions with other antigens (Lucero *et al.,* 1999).

1.6.4 Culture of *Brucellae*

Brucellae must be handled in level 3-laboratory containment facilities due to its potential to cause infection in laboratory workers (Hall, 1990). When *B. abortus* infection is a possibility, incubation should be in an atmosphere with 5-10% CO_2. Blood cultures should be maintained for at least 8 weeks before they are discarded as negative. Isolation rates can be markedly improved if materials from bone marrow or liver biopsy are also cultured (Corbel *et al.,* 1989a).

In the past *Brucellae* used to be isolated by inoculation into guinea pigs, but now this method is carried out only when the sample is heavily contaminated (Bercovich *et al.*, 1996). The isolation of *Brucellae* from milk, dairy products or veterinary material usually requires selective media. Various selective media for culture of *Brucellae* have been used including those described by: Kuzdan and Morse (1953), Meyer (1974), Farrel (1974) and Leech *et al.*, (1984). Comparative trials have shown that these are a satisfactory alternative to guinea-pig inoculation methods (Hunter and Kearns, 1977).

1.7   The viability of *Brucellae* and antibacterial sensitivity

Brucellae are killed after heating at 60°C for 10 minutes and are therefore killed in milk by pasteurization at 72^{0}C. *Brucellae* are moderately sensitive to acid and die out in most cheeses undergoing lactic acid fermentation. They are very sensitive to sunlight but may persist in soil or dust for 2-3 months and for longer in dead foetal material, especially at low temperatures (Young, 1995a). They are sensitive to many disinfectants and antibiotics, including ampicillin, co-amoxiclav, cephalosporins, aminoglycosides, tetracyclines, chloramphenicol, ciprofloxacin, sulphonamides and cotrimoxazole; they are relatively resistant to vancomycin, nalidixic acid and polymyxins (Young, 1995a).

1.8   Brucellosis in humans

B. melitensis is the most common species infecting man and is probably the most virulent, followed by *B. suis* and *B. abortus* (Domenech *et al.*, 1983). In humans, brucellosis is typified by a wide range of clinical manifestations and can occur clinically as an acute, sub-acute, chronic or subclinical disease. In addition, those repeatedly exposed to the pathogen (such as veterinarians), may develop a hypersensitivity state,

shown, for example, by the development of rashes on the arms after examining cows (Trunnell *et al.*, 1985; Mazokopakis *et al.*, 2003).

Human brucellosis is notoriously a multisystemic disease with varied manifestations; the onset may be either acute or insidious. The latter mode of presentation causes more difficulties in diagnosis (Andriopoulos *et al.*, 2006; Young *et al.*, 2000b). Acute brucellosis occurs mainly with *B. melitensis* infection and a minority of cases of *B. suis* and *B. abortus* infection. The predominant clinical features of brucellosis include high fever, headache, malaise, drenching sweats, fatigue and joint pain. Occasionally there is splenomegaly and slight liver enlargement (Fallatah *et al.*, 2005; Troy *et al.*, 2005). Other rare serious complications of brucellosis include, endocarditis, thrombophlebitis, meningo-encephalitis, neuropsychiatric features such as depression and chronic arthritis which can affect the spine (Charters, 1980; Fallatah *et al.*, 2005). Acute brucellosis may last for a period of 2 –3 weeks, and the chronic form up to one or two years, during which the patient may be severely debilitated (Hurvell *et al.*, 1971). The case-fatality rate of untreated brucellosis is 2% or less (Jacobs *et al.*, 1990).

Humans usually become infected with *Brucellae* through direct contact with infected animals or their products. Unpasteurized milk and processed dairy foods from infected animals are the major source of infection for the general population (Young, 1995b). In man brucellosis was initially linked with drinking unpasteurized goat's milk but later it became clear that any contaminated animal product could be the source of infection, either by contact or by consumption (Mishal *et al.*, 1999; Karimi *et al.*, 2003). Other routes of less importance include absorption via the mucous membranes of the respiratory tract or conjunctiva (Hungerford, 1975).

1.9   Risk factors for disease transmission to humans

Transmission of brucellosis to humans requires a close or direct contact with infected animals and animal products. Risk factors for human disease are therefore expected to relate to occupational hazards, and patterns of consumption of animal products (Corbel, 1997). In Kuwait brucellosis as an occupational disease was found to be more common in males than females and occurred in the ages 20-40 years (Lulu *et al.*, 1988). Infection was mainly found to be acquired through handling infected animals at calving or abortion and handling carcasses or offal of infected animals. Infection in the general population was found to be acquired through ingestion of contaminated dairy products especially unpasteurized milk, creamy soft cheese and ice creams (Lulu *et al.*, 1988).

Unpasteurized milk and processed dairy foods from infected animals are the major source of infection for the general population, and infected carcasses are the source of infection for workers in the meatpacking industry (Busch and Parker, 1972). Veterinarians can acquire brucellosis as a result of assisting births of infected animals, as well as through inadvertent exposure to vaccines. Airborne transmission of bacteria to humans has also been documented in clinical laboratories and abattoirs (Buchanan *et al.*, 1974). In Iran, positive results were found by RBPT, SAT titre (1:80) and 2ME titre (> = 1:20) in those engaged in slaughter of animals (10%, 20% and 6% respectively), butchers (6%, 4% and 1% respectively) and the general population (1%, 2% and < 1% respectively) (Karimi *et al.*, 2003). In South Africa, 72% of occupational infections of abattoir workers were found to be due to *Brucellae species* (Mauff, 1980). The same proportion was attributed to brucellosis amongst veterinarians and farm workers in Ethiopia (Seboxa, 1982).

1.10   Under-diagnosis of zoonoses

In Africa, most human diseases are frequently under-diagnosed, particularly among the poor, reflecting the limited capacity and coverage of the health services. However, in the case of the zoonoses, this problem of under-diagnosis is further aggravated by uneven geographical distribution of the poor and the inherent difficulties in diagnosing some of these diseases (Schwabe, 1984). The low priority accorded to zoonoses, and difficulties in diagnosis and hence treatment has made the ultimate outcome for the poor infected with zoonotic diseases to be even worse. The under-diagnosis of zoonotic diseases has greatly contributed to their under-reporting in sub-Saharan Africa countries. Official figures of rabies, brucellosis and trypanosomiasis incidences represent only a fraction of the actual disease burdens in these countries (WHO 1998b; Cattand *et al.,* 2001; Cleaveland *et al.,* 2002).

1.11   Burden of brucellosis

As described earlier, most zoonoses such as brucellosis are under-diagnosed in Africa. Although human brucellosis is a modifiable disease in many countries, official figures do not fully reflect the number of people infected each year, and the true incidence has been estimated to be between 10 and 25 times higher than reported figures indicate (WHO, 1997). Cases very often remain unrecognized because of inaccurate diagnosis, and are thus treated as other diseases or as "fever of unknown origin" (Colmenero, *et. al.*, 1996). Animal brucellosis also poses a barrier to trade of animals and animal products and could seriously impair socio-economic development, especially of livestock owners, the most vulnerable sector in many rural populations (WHO, 1997).

Brucellosis causes production losses in infected herds, both as a result of abortions in newly infected herds, and as a result of a high incidence of retained placenta and endometritis (Corbel *et al.*, 1989a). Brucellosis causes financial losses due to the costs of animal treatment, reduced number of live calf births and concurrent human infection. Humans succumb to prolonged periods of general body malaise, headache, backache, fever and chills (Young *et al.*, 2000b). Further, financial resources are required to cater for human treatment making the economies of the affected people, who are mainly the poor, to be even worse.

Official estimates put losses due to brucellosis in Latin America equivalent to about US$ 600 million annually, which explains the priority given by animal health services to reducing the incidence of the disease (WHO, 1997). The World Health Organization reports an annual incidence of brucellosis in humans of 1 to 78 cases per 100,000 population in the Middle East, with six countries reporting an annual total incidence of over 90,000 cases. Among the Latin American countries, Argentina reports the largest incidence, followed by Mexico and Peru (WHO, 1997).

In Mongolia Roth *et al.*, (2003) conducted an analysis of the economic benefits, cost-effectiveness, and distribution of benefits as a result of mass vaccination of cattle. Results indicated that if the costs of vaccination of livestock against brucellosis were allocated to all sectors in proportion to the benefits, the intervention might be profitable and cost effective for the agricultural and health sectors. So the benefits of the disease control need to account for dual benefits to public health and livestock sectors.

1.12   Brucellosis in animals

Brucellosis in animals is considered to be transmitted mainly through ingestion of the organism from contaminated pastures and water (Blood and Radostits, 1989). Transplacental transmission can occur in calves born to infected dams (Crawford *et al.*, 1986). Cows occasionally may be infected by coitus, licking or when inseminated artificially with infected semen. Abortion, stillbirth, metritis, placentitis and infertility characterize *Brucellae* infection in cattle (Hungerford, 1975).

Several factors have previously been identified as risk factors for brucellosis including, environmental factors such climate, altitude, biotype, herd size, age of cattle and husbandry methods, which play an important role in changing the epizootological pattern (Mikolon *et al.*, 1998; Blood and Radostits, 1989). In eastern and western Uganda, a study conducted on goats randomly selected from 145 herds showed that free browsing when compared to tethering or zero-grazing, and lack of veterinary care were the most-important factors identified for *B. melitensis* herd seropositivity (Kabagambe *et al.*, 2001). In Mexicali Valley, California, risk factors for brucellosis seropositivity of goats in herds were identified as importation of goats from other Mexican states, the presence of La Mancha breed does, and the presence of does born outside the herd. Increasing herdsize was also highly significant. In addition, a significant positive association was found between the presence of seropositive dogs (as assessed by the RBPT) and seropositive goats on the same ranch (Mikolon *et al.*, 1998).

1.13   Wildlife and their role in zoonoses transmission

Diseases transmitted between humans, wild and domestic animals have important impacts on public health, wildlife conservation and livestock economies (Cleaveland *et al.*, 2001; Alonso and Starkey, 1994). Some examples of diseases that affect both domestic and wild animals are: classical swine fever (hog cholera) in wild boars and domestic swine; myxomatosis and rabbit hemorrhagic disease in domestic and wild rabbits; bovine viral diarrhoea (BVD) in cattle and roe deer; contagious ecthyma in domestic sheep and goats and also in, e.g., chamois, muskox, and reindeer; *Mycobacterium bovis* in cattle, wild boars, badgers, and deer; and brucellosis in a broad range of livestock and wildlife (Frolich *et al.*, 2002).

Over a quarter of pathogens of humans and domestic mammals have a very broad host range and are capable of infecting humans, domestic and wildlife hosts (Cleaveland *et al.*, 2001). If such diseases can spread within human populations or if they spread frequently from animal reservoirs, they have serious socio-economic impact (Bengis *et al.*, 2002). There is, however, difficulty in managing wild populations and their diseases in national parks and other protected areas. In some instances, human intervention may be justifiable in order to protect native populations, domestic animals, and humans from acquiring a disease (Alonso and Starkey, 1994). Brucellosis has been known to exist in populations of wildlife since the early part of the 20th century. In the US, brucellosis has been virtually eliminated in domestic livestock after decades of expensive governmental disease prevention, control and eradication programmes. Now the most likely source of transmission of brucellosis to humans, and the risk of reintroduction of brucellosis

into livestock is from infected populations of free-ranging wildlife (Davis and Elzer, 2002).

Studies on brucellosis in African game and cattle living in close contact were conducted by Roth (1967) in Rhodesia, Sachs *et al.,* (1968) in Tanzania and Schiemann and Staak (1971) in Uganda. All these authors were able to isolate species of *B. abortus* and *B. melitensis* from wildlife; they reached a conclusion that wildlife might be the potential reservoirs of brucellosis in Africa. In Tanzania, Mlengeya *et al.,* (personal communication) found out that 41/103 (39.8%) of buffalo sampled in the Arusha region tested positive for brucellosis using RBPT. In a study conducted by Shirima *et al.,* (2003), it was established that out of 90 wildlife sera tested for brucellosis using the RBPT, 13 % were positive for brucellosis. These included 10% of wildebeest, 28% buffalo and 13% impala. The presence of *Brucellae* in wildlife indicated that wildlife could also play an important role in the transmission of brucellosis to domestic animals and humans in a place where they are in close contact.

1.14   Treatment of brucellosis

Although *Brucellae* are susceptible to antibiotics, human brucellosis continues to pose a therapeutic problem because of the intracellular localization of the *Brucellae* within the host's reticulo-endothelial cells, a site relatively inaccessible to antibiotics (Khan *et al.,* 1989; Hall, 1990). Inappropriate choice, dosage and length of antimicrobial therapy, failure of patients to take prescribed drugs and, very rarely, antibiotic-resistant *Brucella* strains are associated with unpredictable relapses after treatment. Hence, the institution of a proper combination of

antibiotics for longer periods is warranted to improve the outcome and prevent relapses (Ariza et al., 1985).

Brucellosis therapy is related to the stage of the disease. In the acute stage, antibiotics can achieve good therapeutic results, and in the chronic stage, additional symptomatic treatment with special emphasis to neurological and psychiatric management can be used with excellent results. Early diagnosis is important so as to reduce severe disability and loss of work ability (Olszok and Kucharz, 1994). Tetracyclines alone are effective for treatment of brucellosis, but the combination of one of the tetracyclines e.g. doxycycline, with streptomycin is currently regarded as the best treatment to reduce the relapse rate for patients receiving antibiotic combinations (Hall, 1990). The combination of cotrimoxazole with rifampicin or tetracycline and streptomycin with rifampicin is also effective (Hall, 1990). Most strains of Brucellae are highly resistant to the penicillin and cephalosporins. Kanamycin and gentamicin can replace streptomycin if resistance develops (Hall, 1990).

Effectiveness and therapeutic value of doxycycline plus streptomycin, and doxycycline plus rifampicin for the treatment of human brucellosis were assessed by Colmenero et al., (1989). The tolerance to both regimens was found to be good, but the combination of doxycycline plus rifampicin offered a more convenient oral administration (Colmenero et al., 1989).

In a study by Ariza et al., (1995) to establish the risk factors associated with relapse of brucellosis, it was found that "less-effective" antibiotic therapy, positive blood cultures during initial disease, being a male, and low platelet count were the

main factors associated with relapse. However, these data showed that relapse of brucellosis is sometimes difficult to diagnose and that it can be an insidious disease. In addition to inappropriate antibiotic therapy, other factors, such as those indicating a more aggressive disease and/or a deficient immunologic response, seem to play an important role in the relapse of brucellosis (Ariza *et al.,* 1995).

1.15 Brucellosis and HIV

As a systemic disease, HIV/AIDS presents with multiple clinical features, some of which resemble brucellosis. A study conducted in Spain by Moreno *et al.,* (1998) showed that HIV infection does not seem to increase the incidence of brucellosis because most cases of brucellosis were found to occur in asymptomatic patients with relatively preserved immunity. It was therefore concluded that the epidemiology, clinical presentation, diagnosis, response to therapy, and outcome of brucellosis cases are similar to those observed in non-HIV infected patients. In a study conducted in Kenya by Paul *et al.,* (1995) it was established that there was no association between *Brucella* antibody status and HIV status. It was therefore concluded that *Brucella* serology may be helpful in the diagnosis of patients with non-specific symptoms in East Africa, regardless of their HIV status. However relapse in brucellosis was found to be related to the profound impairment of the immune response induced by HIV infection, so HIV infection must be excluded in patients with relapsing brucellosis in areas with a high prevalence of both infections (Ibarra *et al.,* 2003).

1.16   Brucellosis in different parts of the world

1.16.1 Brucellosis in Europe, America, Asia and Australia

Brucellosis is more common in countries that do not have good public health and
domestic animal health programmes (World Bank, 1985). Areas currently listed as
high risk are the Mediterranean basin (Portugal, Spain, Southern France, Italy,
Greece, Turkey, North Africa), South and Central America, Eastern Europe, Asia,
Africa, the Caribbean, and the Middle East (World Bank, 1985).

Brucellosis was once a major disease in developed countries, but has now been
eradicated and controlled in several countries through strict control regimens that
include pasteurization of milk, test-and-slaughter policy, vaccination, monitoring
and restriction of animal movements (Meldrum, 1970). For instance, the
prevalence of brucellosis in Greece was dramatically reduced by an eradication
programme, which included test and slaughter (Avdikou *et al.,* 2005). In New
Zealand, because of the high prevalence of the disease, mass vaccination of calves
was employed prior to a test and slaughter policy (Adlam, 1978). In instances
where the prevalence rate was low, mass test and slaughter was often the first line
of action. Prevention of human infection was possible through mass eradication,
protective measures against contact with infected animals, animal products,
aborted materials and pasteurization (Young *et al.,* 2000a).

The incidence of brucellosis caused by *B. melitensis* in sheep, goats and humans is
still a very significant problem in Macedonia and Greece. The disease is an
endemic problem in some regions of the former Yugoslavia and includes *B. suis* in
pigs and in Croatia, *B. melitensis* in sheep, goats and humans is found occasionally
(Taleski *et al.,* 2002). In India brucellosis was first recognised in 1942, but it is

now endemic throughout the country. However, there are no organized and effective brucellosis control programmes in India (Renukaradhya *et al.*, 2002).

The epidemiologic pattern of human brucellosis in the United States since the early 1970s may have shifted from an occupation-associated disease involving *B. suis* to one more common in the general population (Alonso and Starkey, 1994). This change may be attributed to the swine brucellosis eradication programme implemented in 1961, and increased reporting of human *B. melitensis* infection, which was considered to have been eradicated from U.S. sheep and goats in 1972. Hispanic populations of California are at increased risk for *B. melitensis* infection, with imported soft cheese the most commonly reported vehicle of exposure (Alonso and Starkey, 1994).

1.16.2 Brucellosis in Africa

Human brucellosis has been reported in many African countries with higher prevalence around the Mediterannean basin. Living in close contact with cattle exposes people who practice nomadic type of life-style to the high risk of brucellosis. Nomads move with their cattle long distances and in so doing, the animals are liable to acquire infection from a wide range of pastures. The infection is then easily transmitted to humans due to different traditional practices (Cox, 1966). The habit of eating raw meat, e.g. raw liver or other offal with spices (*Marrara* or *umfitfit*) was found to be an important epidemiological factor in contracting the disease in central Sudan. In this area the majority of patients were found to have a combined infection of both *B. abortus* and *B. melitensis* (Mohd, 1989).

Brucellosis has been reported in Kenya since 1913 (Barnett, 1950). A study conducted in Kenya by Oomen (1976) showed that the most prevalent species of brucellosis was *B. melitensis* and human brucellosis showed a tendency to run a chronic course with most cases showing spine and hip joint involvement. Amongst patients recorded with flu-like symptoms (n= 1,0337,875) in Narok hospital, Kenya, between 1986 and 1992, 79.3% were diagnosed as suffering from malaria, 7.1% rheumatism, 2.4% pyrexia of unknown origin (PUO) and 0.8% were diagnosed as suffering from brucellosis (Muriuki *et al.*, 1997). The coexistence of diseases which present with similar non-specific symptoms such as malaria, syphilis, PUO and tuberculosis has been causing difficulties in the diagnosis of brucellosis in many parts of Africa (Colmenero, *et al.*, 1996; Young, 2000a).

In Uganda, Ndyabahinduka and Chu (1984) found that brucellosis was widespread in the country. Positive agglutinin reactors among hospital patients in endemic areas formed 18-24% of the total tested. A study conducted by Galukande, (2005) showed that brucellosis contributed to serious spinal pathology in 17.2% of 204 patients who presented to Mulago hospital in Uganda with lower back pain. When multiple tests were requested on patients having general malaise or body joint pains and/or constant headaches (n=105), malaria was found to play a major role (73%) followed by brucellosis (13.3%) and syphilis (4.3%) (Mutanda, 1998).

1.16.3 Brucellosis in Tanzania

In Tanzania, brucellosis was first diagnosed in animals in 1928 from samples taken from aborted cattle at Engare Nanyuki (Kitalyi, 1984). In humans, brucellosis was first reported by Evans (1935) after reviewing the status of the disease in the then Tanganyika. He stated that cultures obtained from humans had shown that *B. abortus* and *B. melitensis* occurred in the country. Further reports of human brucellosis in the country were obtained from monthly reports of the medical department of the Lake Region and west region for 1959, 1960 and 1961 and from two patients at Kihesa village in Iringa region (Anon, 1963). Several surveys have then been conducted on animal brucellosis in different parts of Tanzania, with prevalences varying from 5% to 20% (Table 1.2). However, these studies were conducted in more intensive farming systems, and often in response to abortion problems and may therefore not be representative of the majority of the indigenous cattle population, kept under traditional husbandry systems.

Table 1. 2: Surveys of bovine brucellosis conducted in Tanzania

Region/Zone	Test used	Seroprevalence (%)	Reference
Southern highlands	SAT	13.2	Mahlau and Hammond, 1962
Southern highlands	SAT	20.5	Mahlau, 1967
Arusha and Manyara	SAT	6.9	Staak and Protz, 1973
Central zone – Dodoma	SAT	5.1	Kitaly, 1984
Southern regions of Mtwara, Lindi and Ruvuma	SAT	2-3	Otaru, 1985
Lake zone- Mwanza	SAT	10.6	Msanga *et al.,*1986
Coastal regions -Coast region	SAT	2.3	Weinhaupl *et al.,* 2000
-Dar es Salaam	SAT	14.1	Weinhaupl *et al.,* 2000

The findings are evidence that brucellosis has been in Tanzania for a long time at varying degrees, however, despite this no clear control strategies have been put in place. Currently the burden of human brucellosis in Tanzania is not known. A need to identify and quantify the burden of brucellosis, the relative importance of different risk factors, as well as evaluating diagnostic tools for brucellosis in Tanzania form the basis for this study. Although a number of studies of human brucellosis have been carried out in Tanzania, none has explored the relationship between infection in humans and animals in different communities.

1.17   Control of brucellosis

The control measures of brucellosis in most African countries rely on public awareness, campaigns on the importance of boiling milk and better diagnosis of

human cases which are rarely implemented because health policy makers are not aware of the burden imposed by zoonoses such as brucellosis (Coleman, 2002). Control and eradication of brucellosis in domestic animals has important public health implications. Test-and-slaughter programmes, in conjunction with vaccination, are the major method of control (Crawford *et al.*, 1986). Whole herd depopulation can also be used when other methods have reduced disease prevalence to low levels. Livestock populations can be screened for brucellosis by serologic testing of individual animals, or by testing pooled samples such as bulk milk (Crawford *et al.*, 1986).

Several vaccines are available for reducing infection in animal populations, thereby reducing transmission potential to humans (Olsen and Stoffregen, 2005; Crawford *et al.*, 1986). These include, S19, a laboratory-derived strain containing *B. abortus* and Rev1, a live attenuated *B. melitensis* vaccine. S19 induces reasonable protection against *B. abortus*, but produces persistent serological responses which mimic active infection during testing (Schurig *et al.*, 2002). A similar problem occurs with the Rev1 strain which is still the most effective vaccine against caprine and ovine brucellosis (Schurig *et al.*, 2002; Mahajan *et al.*, 2005). RB51 is a mutant *B. abortus* strain vaccine, which has proved safe and effective in the field against bovine brucellosis, and exhibits negligible interference with diagnostic serology; it allows the differentiation of vaccinated and infected cattle by means of serological tests (Moriýon *et al.*, 2004).

The efficacy of the RB51 vaccine for the collective prophylaxis of brucellosis in ruminants is controversial (Moriýon *et al.*, 2004) and moreover, there is a lack of

interest in this vaccine in countries which do not require differentiation of vaccinated and infected animals. The classical live and attenuated *B. abortus* S19 vaccine is therefore considered to be the best available vaccine for controlling brucellosis in cattle (Plommet and Plommet, 1989).

In many cases the control measures that have worked well in the developed world can not be easily applied in developing countries where people depend on their animals for their livelihood and replacements are difficult to obtain (Coleman, 2002). On the other hand, local customs, traditional habits and beliefs have been impeding the wide application of such measures. Health education should be intimately linked with all phases of prevention and control activities for zoonoses (WHO, 1997). Protective clothing and careful handling of infected animals have all been suggested by different studies as methods to reduce occupation-related brucellosis and avoidance of infection in the general population as a whole (Buchanan *et al.,* 1974).

1.18 Rationale and objectives of the study

1.18.1 Rationale

The study was motivated by members of the public and hospital staff in Arusha and Manyara regions while conducting a study on tuberculosis. A significant number of community members cited brucellosis as the most common disease in their households after malaria. In hospitals, practitioners admitted to seeing a significant number of patients with brucellosis in every single day. However, it was evident from the visits that there were problems related to lack of standardized diagnostic and treatment protocols for brucellosis which needed to be studied and resolved.

Very few studies have been conducted on human brucellosis in Tanzania. This was supported by lack of adequate data on human brucellosis in Tanzania. Most of the studies conducted on brucellosis in Tanzania focused on quantifying the magnitude of animal brucellosis in areas where animal abortions have been reported, and mainly in more intensive farming systems instead of the majority indigenous cattle population kept under traditional husbandry systems. Further more, none of the studies used a population based random sample.

Tanzania is predominantly an agricultural country and the agricultural sector has been identified as a key industry for development and poverty alleviation. The livestock sector, which is part of the agriculture sector, forms an integral part of the Tanzanian economy (Ministry of Agriculture and Cooperatives,

1995a). As the future lies mainly in agricultural development, diseases with considerable effect on livestock productivity such as brucellosis should not be ignored (Stark and Protz, 1973). Brucellosis is known to contribute to poverty not only as a constraint to the productivity and development of the livestock sector, but also by causing a direct human health burden (Stark and Protz, 1973). The present brucellosis control strategies need to be reviewed with the establishment of a properly- planned, clearly defined and well enforced policy. The public should be made aware of the seriousness of the disease and its zoonotic potential so that participation in control strategies can be easier. It was, therefore, felt that there was a need to conduct an epidemiological study on brucellosis that would address several key questions such as those related to diagnosis and treatment, risk factors for brucellosis, health seeking behaviour and clinical characteristics of brucellosis cases and the burden caused by brucellosis in Arusha and Manyara regions.

According to the livestock census of 1994/95, Arusha and Manyara regions are among the regions keeping the majority of livestock in Tanzania. The regions have the majority of pastoralist communities such as Maasai and Barbaigs, and many agropastoral communities. The regions harbour the Ngorongoro and Tarangire National Parks and border the Serengeti National Park and other extensive lands of free-ranging wild animals. The people living in Arusha and Manyara regions are also known to keep other animals such as dogs and pigs. Both wildlife and domestic animals have been shown to be reservoirs of brucellosis (Roth, 1967; Sachs *et al.,* 1968; Schiemann and Staak 1971). The study area therefore provided an opportunity to study the role of

farming systems, wildlife and domestic animals in the transmission, risk factors and other important factors related to brucellosis.

1.18.2 The objectives of the study were:

- To determine the sero-prevalence and incidence of human brucellosis in Arusha and Manyara regions, Tanzania.

- To determine the burden caused by brucellosis in Arusha and Manyara regions, in terms of DALYs lost and the economic burden associated with the disease and its treatment in human populations.

- To determine the clinical history and health-seeking behavior of brucellosis cases in Arusha and Manyara regions.

- To determine the knowledge of medical practitioners relevant to the diagnosis of common zoonotic diseases.

- To identify and quantify the risk factors for brucellosis in humans.

- To evaluate the efficacy of different tests used in the diagnosis of human brucellosis in field and hospital settings and to investigate the implications of misdiagnosis.

2. Materials and Methods

2.1 Introduction

Tanzania is divided into 26 administrative units called regions. Each region is then divided into districts, divisions, wards, villages, sub-villages, ten-cell units and finally households. Arusha and Manyara regions were involved with the study on tuberculosis which was conducted prior the current study. Sampling was therefore convenient in that it was easier to involve the same community, veterinary, hospitals and political staff with the current study. Arusha districts include Arusha, Karatu, Monduli, Arumeru and Ngorongoro and Manyara districts include Babati, Mbulu, Kiteto, Simanjiro and Hanang. The study involved Karatu, Ngorongoro, Babati, Hanang and Mbulu districts (Figure 2.1).

Four study designs were employed in this study. These included the retrospective hospital baseline data collection (preliminary hospital survey), the community (cross-sectional) survey, the prospective hospital survey and the matched case-control study. Baseline data for brucellosis were obtained from the preliminary hospital survey and data for health-seeking behaviour and clinical characteristics of brucellosis cases, evaluation of diagnostic tests, knowledge of practitioners of zoonoses and estimation of burden caused by brucellosis were obtained from the prospective hospital survey. In the case-control study, cases established in the prospective hospital survey and the controls matched to cases by sex and districts were followed up to their homesteads for blood sampling and questionnaire administration.

2.2   Study area and hospitals involved

Arusha and Manyara regions are located in the north area of Tanzania. Arusha and Manyara regions formed a single region called Arusha prior to 2002. The regions lie between 1.8^0 and 6^0 south of the equator and between 35^0 and 40^0 east of Greenwich Meridian with a large area of landscape more than 1000m above sea level. Most of the areas receive substantial rains from March to May, and lighter rains from September to December, with the rest of the year being dry, typical of a semi arid area. The major ethnic groups in Arusha and Manyara regions include Maasai, Mbulu (Iraqw), Barbaig, Fyomi and Sonjo.

Maasai and Barbaigs are primarily livestock keepers, practising traditional pastoralism and following a semi-nomadic lifestyle. The predominant form of land-use among the other ethnic groups is agropastoralism with people keeping livestock, but also growing crops for subsistence. People from these ethnic groups are more settled, do not move far with cattle in search of pastures and have more permanent homesteads. Although ethnic groups are not found exclusively in one district, the Maasai and Sonjo predominate in much of Ngorongoro district, the Barbaig live mainly in Hanang, the Fyomi in Babati and Hanang districts, and the Iraqwi in Karatu, Babati and Mbulu districts. In total the study districts have 285 villages with a total population of 1,054,238 people.

Each district has either a district or a designated district hospital. District hospitals involved with the study included Babati hospital in Babati, Mbulu hospital in Mbulu and Katesh hospital in Hanang. The designated district hospitals included Dareda hospital in Babati, Karatu Lutheran hospital in Karatu and Wasso hospital in Ngorongoro. Although Endulen and Hydom hospitals are neither district nor designated district hospitals, they

serve large catchments area in Ngorongoro and Mbulu districts respectively (Figure 2.2). The majority of patients in the study area go to district or designated district hospitals rather than dispensaries because the hospitals are better equipped and staffed than dispensaries.

Figure 2. 1: Map of Africa showing the location of Tanzania and the study area
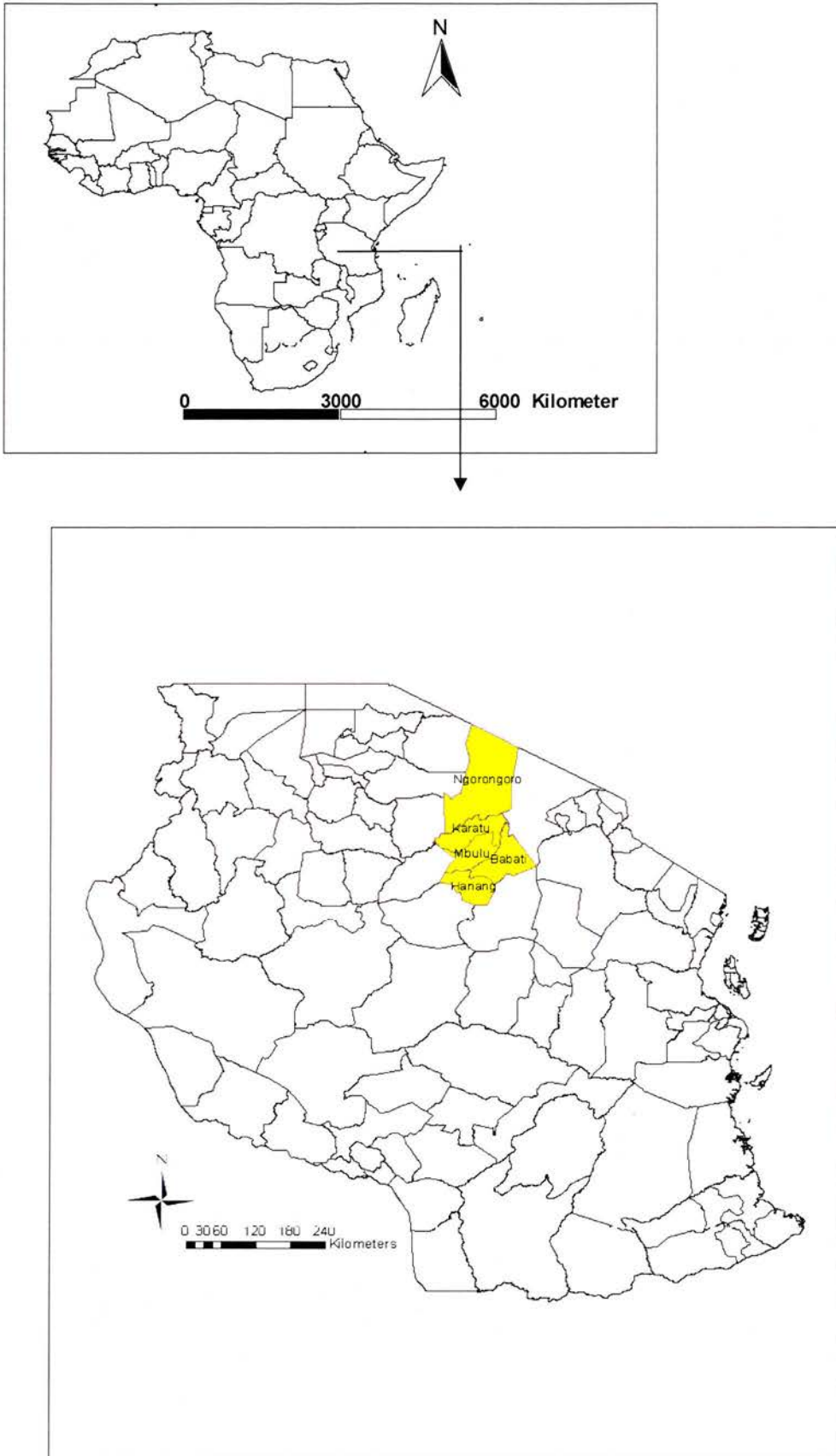
Figure 2. 2: Locations of the hospitals in the study
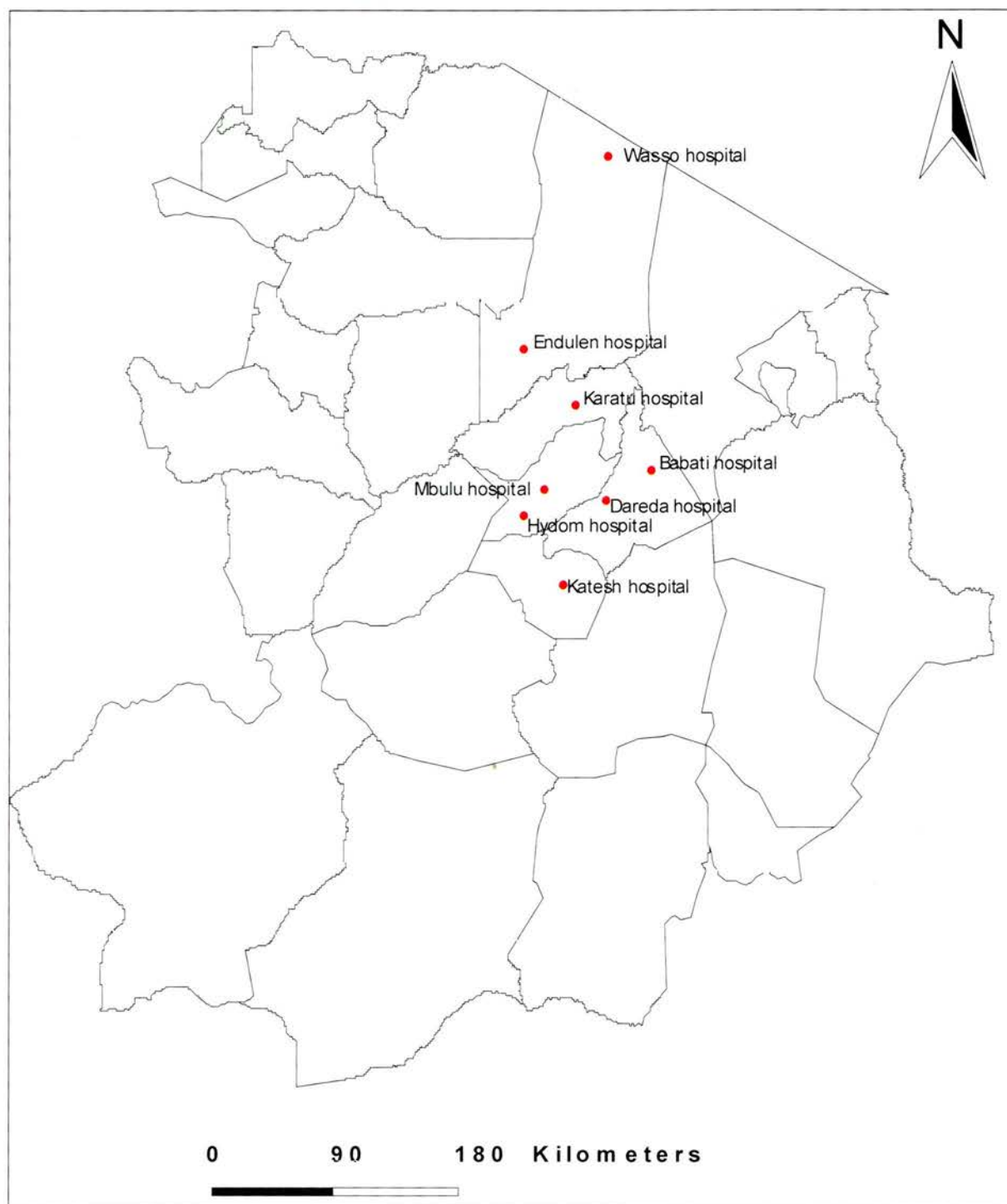
2.3 Preliminary hospital survey

The preliminary hospital survey was conducted between July and September 2002. Data of the year 2001 from all the hospitals involved in the study were recorded and interviews with practitioners of the hospitals were conducted to establish baseline information on brucellosis in the study area. The objective of the study was to establish baseline information on brucellosis in Arusha and Manyara regions, including diagnostic and treatment protocols for brucellosis, the proportion of patients diagnosed and treated for brucellosis at the hospitals, geographic distribution and socio-demographic characteristics of brucellosis patients over one year, and the costs for investigation and treatment charged by the hospitals.

In total, 11 practitioners agreed to be interviewed by the principal investigator of the study. These included one medical officer and 10 medical assistants (see section 9.3.1 for the level of training that each receive). Four questions were asked during the interview, these included the types of drugs used for the treatment of brucellosis, diagnostic criteria for brucellosis, whether respondents had seen relapse or chronic brucellosis patients and their opinions on when most patients presented to hospital.

As the hospitals visited did not store their data in electronic form, files with data of the year 2001 were reviewed and all data recorded. These included data on all the patients who attended to the hospitals during the study period, the number of brucellosis patients, where they were coming from, when they were diagnosed and the treatment they received. The costs of consultation, drugs and investigations were also recorded.

2.4 Cross-sectional survey

2.4.1 Household cross-sectional survey

The cross-sectional study was conducted between May 2002 and July 2003. The households to be visited were selected by a multistage cluster sampling using a table of random numbers. The sampling frame comprised all of the 285 villages in the study area. From the villages, a sample of 32 villages comprising 20 pastoral and 12 agropastoral villages were randomly selected. In each village, two sub-villages were then randomly selected. From each sub-village, two ten cell leaders were selected randomly and finally two livestock keeping households were selected randomly from a list of all households under each ten cell leader. At the randomly selected households, all members were encouraged to be tested for brucellosis.

The first visit was made prior to blood sampling and delivery of the questionnaire. Consultations were held with household leaders to provide information on the purpose of the study and proposed blood sampling of household members and livestock. Consent was sought for participation in the questionnaire study and for sampling of humans and livestock. So as to motivate members to come forward for blood sampling, they were promised by the research team that results and health education would be given on the same day. Those who tested positive without symptoms were advised to go to hospital as soon as symptoms developed and those with symptoms were advised to go to hospitals for confirmation and treatment. Pain killers were given to those who agreed to be tested and had pain.

2.4.2   Questionnaire data collection

Open and closed ended questionnaires to establish knowledge, attitudes and practices of the people towards brucellosis and other zoonotic diseases were administered by the principal investigator. Respondents were members of households and information was obtained by posing a question that was later discussed by members of the households and a response given by one of them after they had agreed on the answer. In some households, translators had to be used to ease communication between the investigator and respondents. Translators were recruited on the same day the questionnaire was administered and they were advised not to play part in modifying or influencing the type of response given by the household members. Detailed information on questionnaire data, results and analysis have been described by a co-investigator (Shirima., 2005).

2.4.3   Human blood sampling

Samples were collected from household members who volunteered for participation in the study. This was not a random selection; for example, in some households, the head of the household chose those who would be bled, in others some individuals were afraid of being sampled and in others, some volunteers were particularly keen to participate because of a history of clinical disease. Fourteen household leaders did not consent to participate with the study activities; hence another household had to be selected randomly as a replacement.

The site of fore-arm to be bled was thoroughly cleaned with cotton wool soaked with methylated spirit (Bells Chemicals Co. Ltd., Dar es Salaam). Five millilitres of venous blood was then taken from the brachial vein by using a disposable, sterile needle and

added into a plain sterile 5 ml vacutainer (Becton and Dickinson, UK). In some few individuals, bleeding was done at the back of the hand because blood could not be drawn from the brachial vein due to the vein being thrombosed or was not visible and not easily accessible. Human samples were labelled with an identifier number to ensure patient anonymity at the laboratory.

2.4.4 Livestock blood samples

Blood samples were collected from cattle, sheep and goats in the household, with the number of each species sampled being determined according to the sample size needed to detect infection in a herd, using 99% confidence and power of 80% and prevalence of 5% (Martin *et al.*, 1986). Sampling was carried out at dawn before the animals left the boma and animals were sampled from within each age and sex class. Randomisation of sampling was attempted, but this also cannot be considered strictly random as only animals that could be manually caught and restrained were sampled. Thus, particularly difficult animals were excluded from the study. Further details on livestock sampling and results have been described by Shirima *et al.*, 2005.

Animals were restrained within the premises, and on a few occasions a crush was used. Five millilitres of blood were then taken from the jugular vein using a sterile needle and plain vacutainers (Becton and Dickinson, UK) and for identification purposes the animal was ear tagged using a metal tag (Ketchum, UK) that had a unique identifier number for each animal.

2.4.5 Processing and testing blood samples

Both human and livestock blood samples collected in labelled vacutainers were left to settle at ambient temperature for a period of up to 30 minutes. Serum was later separated by centrifugation with a mobile 12 v centrifuge (Vulcan technologies, USA), then split into two aliquots. One aliquot was used at the household for the RBPT (see section 2.4.5.1). Results were conveyed to each subject tested with confidentiality maintained, and animal results were conveyed to the head of the household. Owners were advised that if they considered to sell an animal for meat in an auction or if they wanted to slaughter an animal for meat at home, then a seropositive animal should be considered first because keeping a seropotive animal will result in further transmission of brucellosis. They were also advised that they should not sell the seropositive animal to a neighbour or in an auction for keeping since the animals might transmit brucellosis to other animals. Members were also advised to protect themselves while handling meet of seropositive animals and their meet should be thoroughly cooked to kill the disease causing organisms

The two aliquots were preserved in cool boxes with ice at (-20°C) and one sent to the SUA by using road transport and later to the VLA, Weybridge, the UK by using air transport. In both laboratories, sera were tested using the RBPT (Davies, 1971) see section 2.4.5.1 and the c-ELISA test (Perret, *et al.,* 2001) see section 2.4.5.2.

2.4.5.1 The RBPT

The antigen for the RBPT was supplied by the VLA (Batch number 269 and SG276). The protocol used in this test has been described by Davies (1971): Briefly, 30µl of test antigen were placed beside 30µl of serum on a flat, white transparent surface and

the drops were mixed using a clean piece of tooth pick in a zone of 2 cm in diameter. The slide was rocked gently and the results were read within 4 minutes in good light. One or two observers were used to verify the results. Any slide with a formation of clumps (agglutinates) was taken as positive, while those without evidence of clumps were classed as negative. A two point scale was used to report the positive results as strong positive or weak positive depending on the density of agglutinates.

2.4.5.2 The c-ELISA test

The protocol for the c-ELISA test has been described by Perret *et al.*, (2001). Briefly, a polystyrene microtitre plate, pre-coated with *B. melitensis* lipopolysaccharide with 96 wells was used. In each microtitre well, 20µl of test serum was added in duplicates using a single channel micropipette. Twenty microlitres of positive and negative control antisera from the VLA were then added into six wells each. One hundred microlitres of conjugate buffer was added into all the wells and the plate was covered with a lid and incubated at room temperature for 30 minutes on a rotary shaker at a 160 revolutions per minute. Plates were then rinsed and then thoroughly dried by absorbent towel.

One hundred microlitres of substrate-chromogen solution were then dispensed into each well, covered and left to react for 15 minutes at room temperature. A 100 µl of stopping solution were then added to each well to stop the reaction. Any well that did not show colour change indicated that the test sample was positive. An ELISA reader (Multiscan RC version 6) was used to read the samples within 20 minutes at 450nm filter with no blank. Based on conjugate control the positive/negative samples were determined by a 60% cut–off of Optical Density (OD) (Based on 60% of the mean of

the OD of the 4 conjugate control wells). Any sample with OD less than the value was taken as a positive and more than 60% was taken as negative. If the binding ratio was less than 10, or the mean OD of the 6 negative wells was less than 0.700, or the mean OD of the 6 positive wells was greater than 0.100, the plate was rejected and samples retested.

2.5 Health-seeking behaviour and clinical characteristics of brucellosis cases
2.5.1 Selection of patients and blood sampling

The study group was drawn by prospective hospital survey. All patients presenting to the hospitals between June 2002 and April 2003 were enrolled into the study. Only patients whose homes were in the study area (Mbulu, Babati, Karatu, Ngorongoro and Hanang districts) were included in the analysis.

Following preliminary discussions with practitioners from the participating hospitals, it was agreed that brucellosis should be considered a possible diagnosis for all patients presenting with one or more of the following non-specific clinical features, including fever, headache, joint pain, malaise, backache, fatigue and loss of appetite. For all patients presenting with these clinical features (defined here as 'suspected brucellosis cases' or 'suspects'), detailed data on clinical history, symptoms, impact of disease, location of household and potential risk factors (such as occupation, age, sex) were collected by the practitioners and data obtained recorded on forms designed for the purpose (Appendix II). For each suspected brucellosis case, a blood sample was collected for serological diagnosis of brucellosis (Figure 2.3). Additional laboratory tests for other conditions, such as malaria and typhoid, were also carried out on the basis of the clinical

judgement of the physician. Each patient was given a unique identification number, which was used to identify data on the clinical forms and the blood sample.

2.5.2 Laboratory tests

All the investigations at the hospitals were carried out according to the hospital's standard practices for routine diagnoses. These included serological tests for brucellosis, a blood smear for diagnosis of malaria, and the Widal test for diagnosis of typhoid fever (Schroeder, 1968). For amoebiasis, routine microscopic examination of stool samples was conducted. As X-ray facilities were not available in most of the hospitals, the diagnosis of pulmonary tuberculosis was based on presenting clinical features and microscopic examination of sputum for the presence of acid-fast bacilli. Difficulties were encountered when a cervical lymph node biopsy was taken to investigate tuberculosis. In such a situation, a biopsy had to be sent to Kilimanjaro Christian Medical Centre in Kilimanjaro for histopathology, hence diagnosis was delayed. Results of diagnostic tests and the treatment regime initiated were recorded on the clinical form by the attending physician (Appendix II). All patients with positive serological results for brucellosis conducted at the hospitals were named as 'brucellosis seropositives' to distinguish them from 'suspected brucellosis cases'.

2.5.3 Confirmation and definition of cases

Samples of blood taken from the suspected brucellosis cases were taken to the VLA where the c-ELISA test was conducted (see section 2.4.5.2). In this investigation, all suspects with any two or more of the clinical features including fever, headache, joint

pain, waist pain, backache, malaise, fatigue and tested positive with the c-ELISA test at the VLA were taken as brucellosis cases.

Figure 2. 3: Enrolment, investigation and follow-up of brucellosis suspects
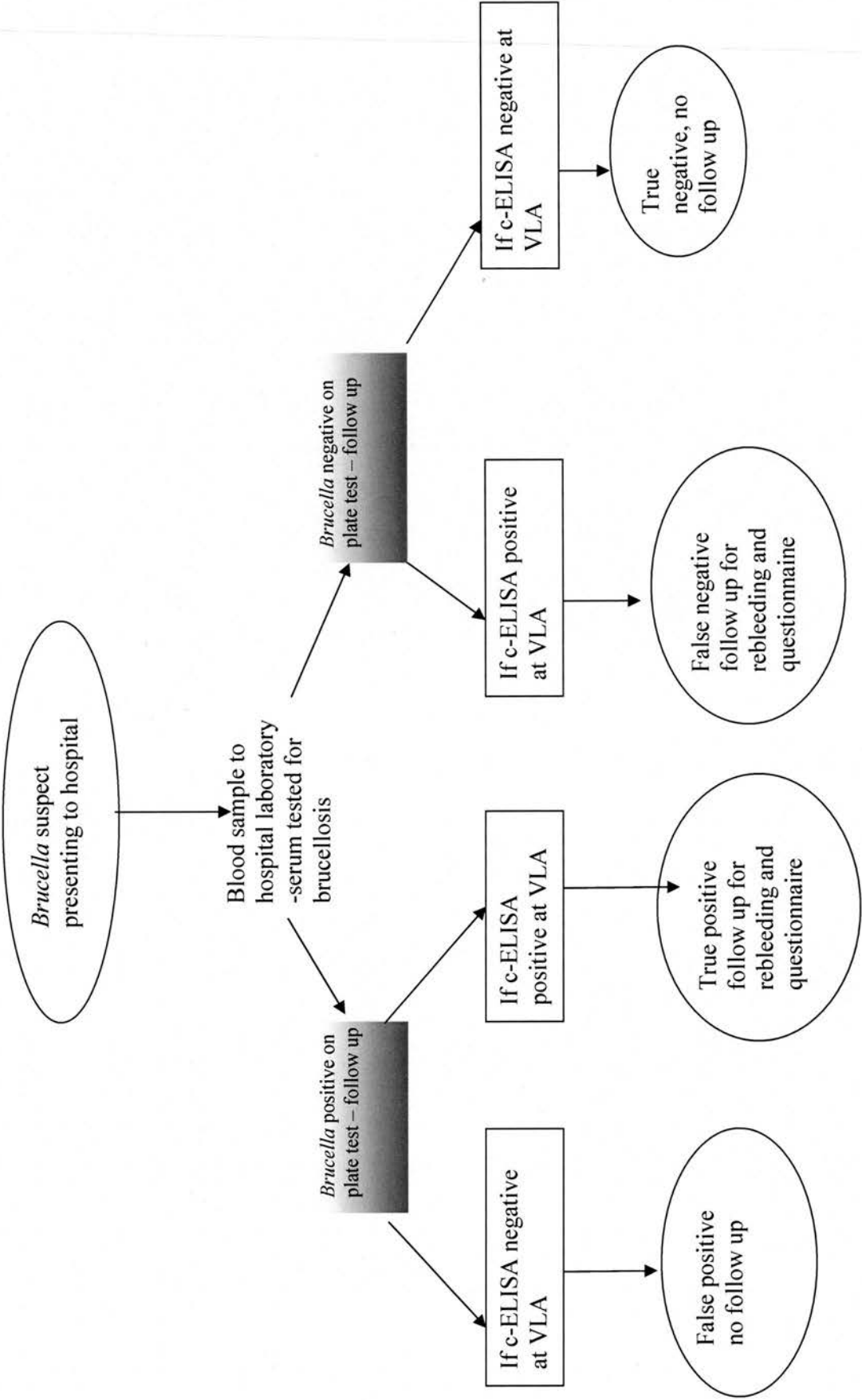
2.5.4 Determination of persistence of antibodies to *Brucella* using the RBPT

Nine cases of brucellosis that were established at hospitals and received treatment for brucellosis were followed-up to investigate the persistence of antibodies to *Brucella*. The objective of the exercise was to determine for what time period cases will continue to test positive for brucellosis. In this part of the study the RBPT was used to determine the presence of antibodies to *Brucella*. The time span between the day treatment for brucellosis commenced to the time the last RBPT positive result was obtained was termed "The duration of antibodies to *Brucella*". Any case who had remission of the original clinical features, established by interview and physical examination, was considered to have recovered from brucellosis.

2.5.5 Calculation of distance between hospitals and households

The GIS coordinates of the households and of the hospitals were recorded from a hand-held Garmin® GPS machine. All coordinates were then transferred to Excel spread sheets and the distance between the hospitals and the households calculated in kilometers using ArcGIS 9 software (ESRI, Redlands, California).

2.6 Case-control study

2.6.1 Study design and case selection

This was designed as a matched case-control study. All patients who presented to the selected hospitals between July 2002 and June 2003 with clinical features suggestive of brucellosis were recorded and investigated. Any person with a positive serological result by

the c-ELISA test conducted at the VLA, and showing at least two of the clinical features: headache, fever which is recurrent or continuous, sweating, joint pain, joint swelling, general body malaise or backache, was defined as a case.

2.6.2 Selection of controls

For every case, a community-based control was selected. A control was an individual in the same district as the case, having a negative brucellosis serological result by the c-ELISA test and matched by sex. To get an appropriate control, a multistage cluster sampling technique was conducted. Random selection was performed at each level that included district, village, sub-village, ten-cell unit and household. As all the cases were drawn from hospitals, the criteria for selection of controls included them coming from a hospital-going household. Any persons from households with a history of member/s going to traditional healers only when falling sick were excluded from the study. To avoid selection of a non-compliant control, all members of each household were encouraged to have their blood taken for brucellosis serology. One control of the same sex as the matched case was selected randomly from among those who agreed to participate in the blood sampling.

2.6.3 Follow-up of cases and controls

Blood sampling and testing for brucellosis in both humans and livestock was conducted using the RBPT in the field and at the SUA in Morogoro (Davies, 1971), the c-ELISA test for brucellosis at the VLA and HIV testing using a direct ELISA test (The Vironostica Uniform II *Plus* O), at the Muhimbili University College of Health Sciences in Dar es

Salaam. A questionnaire on potential risk factors was also administered during the follow-up period (Appendix I). In each household the number of livestock to be bled was determined by using the power of 80% with 95% confidence and prevalence of brucellosis of 5% to detect infection in a herd (Martin *et al.,* 1986).

2.6.4   Laboratory tests

2.6.4.1 The RBPT and the c-ELISA test

The methods involved in the RBPT and the c-ELISA tests have been described in sections 2.4.5.1 and 2.4.5.2 respectively of this thesis.

2.6.4.2 Human Immunodeficiency Virus (HIV) testing

Antibodies against HIV type 1 and type 2 were tested using a direct ELISA test the Vironostica Uniform II *Plus* O test. The kits for the test were supplied by bioMérieux bv of the Netherlands. Briefly, 100 µl of specimen diluent containing stabilizing protein and detergent (gentamicin sulfate) were added by a pipette into all wells of a plate including control wells. Fifty microlitres of test samples were then added by a pipette into all wells except one column which was left for three negative controls and one anti-HIV positive control. Fifty microlitres of negative control was then added into the upper three wells of the last column followed by fifty microlitres of the positive control.

The mixture was shaken by a microshaker for 15 minutes and incubated at 37^0C for 60 minutes. Each well was then washed and soaked with phosphate buffer six times, each

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time the contents of the wells were aspirated into a waste flask and the wells refilled with phosphate buffer. A hundred microlitres of Tetramethylbenzidine in citric acid was added into each well and incubated at 15^0C -30^0C for 30 minutes. To stop the reaction, 100 µl of sulphuric acid was added into each well and the contents mixed by tapping the side of a plate. The reader was then blanked against air and the absorbance of the solution was read at 450nm with 620-700nm as reference

Manually the results were obtained by the following method:

For qualification of Negative Control (NC) values:

1. NC must be <0.250. Eliminate any NC>= 0.250
2. Determine the NC_x value of the remaining controls
3. NC must be within the range of 0.6 to 1.4 times NC_x

Assay validity

An assay was valid if
1. More than half the number of negative controls remain
2. PC1 - NC_x >= 0.400
3. PC2 - NC_x >= 0.400 (if used)

Cut-off point

If the test is valid the cut-off point is obtained by: NC_x + 0.100.

A test sample is reactive if sample absorbance is >= cut-off point and nonreactive if sample absorbance is < cut-off point.

A reactive result indicates that the sample tested either contains anti-HIV-1, anti HIV-2 and or anti-HIV 1 group O, and a non reactive result indicates that the sample tested was negative or contains the above antisera below detectable limits of the test.

2.7 Evaluation of diagnostic tests for brucellosis

2.7.1 Data collection

Serological data for evaluation of diagnostic tests were obtained from both the cross-sectional study and the hospital clinical study. The cross-sectional study was conducted between July 2002 and June 2003. Subjects for blood sampling were obtained by multi-stage cluster sampling from the regional to household levels. At the randomly selected households, all family members were encouraged to be tested for brucellosis (see chapter 3). In the clinical study, all patients presenting with clinical features suggestive of brucellosis (brucellosis suspects) were tested for brucellosis using antigen purchased by the hospital and Rose Bengal antigen supplied by the project. An aliquot of serum was stored for further analysis at the SUA and at the VLA.

2.8 Estimation of the burden caused by brucellosis

Data for DALY estimation, clinical history and costs of treatment were obtained from the follow-up of cases and the cross-sectional survey. Detailed methodology has been described in chapter 8 of this thesis.

2.9 Knowledge of practitioners of common zoonotic diseases

The study was carried out in the districts of Ngorongoro, Karatu and Arusha in Arusha region, Mbulu, Babati and Simanjiro in Manyara region and Moshi in Kilimajaro regions in northern Tanzania, and Dodoma urban in Dodoma region in Central Tanzania. Details of the hospitals involved and methodology applied are discussed in chapter 9 of this thesis.

2.10 Ethical issues

The study was approved by the National Institute for Medical Research (NIMR) ethical committee, and was conducted in accordance to NIMR ethical guidelines. Consent was sought before patients enrolment and before any diagnostic procedure.

2.11 Data analysis

All data were entered and initial analysis done on Microsoft Office Excel 2003 spreadsheets (Microsoft Corporation, Redmond, Washington DC). Different softwares were then used depending on the type of analysis required and data set available. In univariate and multivariate analysis Minitab version 1.4 (Minitab Inc. 2000, Release 14 for Windows, State College, Pennsylvania) was used and Egret for Windows version 2.0 (Cytel Software Corporation, Cambridge Massachusetts) for conditional logistic regression. Chi-square tests were used to analyze all count data and Fisher's exact test was used where 2x2 tabular results produced expected counts of less than 5. A p-value of <0.05 was considered statistically significant.

Egret for windows version 2.0.3 was used to run a conditional logistic regression in the analysis of data for the case-control study. The univariable relationships between all independent variables and brucellosis were estimated by including them individually in a model with brucellosis serological result as the dependent variable. Forty-four risk sets were obtained from the cases and controls studied. Using risk sets as a matching variable, and cases and controls as outcome variables, a number of models were fitted to test their significance as risk factors for a brucellosis positive serological result. Since a few

samples were available for HIV testing, the univariate analysis using HIV results was carried out separately using Minitab version 1.4 and the results were interpreted separately without being included in the multivariate analysis. All the 95% confidence intervals were calculated using Epi Info 6 software (CDC, Antanta, Georgia).

In multivariable analysis, models were created by a backward stepwise procedure. Variables that had a p-value of ≤ 0.2 from the univariable analysis were considered for inclusion in the final models. Values were retained if on their removal there was a significant increase of the residual deviance of the model with likelihood ratio statistics (LRS) of $p>0.05$ and they were removed from the model if they caused an insignificant increase or decrease in the residual deviance with LRS of $p < 0.05$.

In the evaluation of diagnostic tests for brucellosis, Win Episcope 2 (CLIVE, Learning Technology Section, College of Medicine and Veterinary Medicine, University of Edinburgh) was used to draw a Receiver operating characteristic (ROC) curve and the cut-off point with the best results of both Se and Sp was calculated using the method by described by Hanley and McNeil (1982).

2.12   Questionnaires

Questionnaires have been described in respective chapters and included in appendices 1-V. In summary, all the questionnaires were a combination of closed and open-ended questions in English. Interviews were however conducted by the principal investigator in

Swahili which is the language spoken in most parts of Tanzania. In some areas where

Swahili was not commonly used, an interpreter was used.

3. Preliminary hospital survey

3.1 Summary

Objective: The objective of the study was to establish baseline information on brucellosis in Arusha and Manyara regions, including diagnostic and treatment protocols for brucellosis, the proportion of patients diagnosed and treated for brucellosis at the hospitals, geographic distribution and socio-demographic characteristics of brucellosis patients over one year, and the costs for investigation and treatment charged by the hospitals.

Methodology: A review of hospital records and interviews with hospital medical staff were conducted in Arusha and Manyara regions between July 2002 and September 2002.

Results: Of 170,345 patients who attended hospitals in Babati, Dareda, Karatu, Hydom, Wasso, Endulen and Mbulu hospitals in the year 2001, 619(0.36%) were diagnosed as having brucellosis. The majority of brucellosis cases were in the age group 16-30 years compared to other age groups. Different hospitals used several different antigens and techniques to test for brucellosis including titration method and serum/rapid agglutination tests. There was a wide disparity in treatment protocols. Drugs used for the treatment of brucellosis differed amongst the hospitals with respect to doses and duration of treatment.

Conclusion: Evaluation of brucellosis treatment regimes and diagnostic tools with an aim of establishing and hence adopting standardized treatment and diagnostic protocols should be carried out. The government and hospitals should ensure that the recommended drugs and reagents for the tests are available to the hospitals throughout the year. There is a need to carry out a study that will establish to what

extent brucellosis is a problem, not only to the hospital-attending population but also in the communities in Arusha and Manyara regions. Other areas where research is needed includes risk factors for brucellosis, health seeking behavior and clinical characteristics of brucellosis.

3.2   Methodology

Hospitals involved and the methodology used in this study are described in the second chapter of this thesis. In summary, a review of hospital records and interview with hospital clinical staff was conducted between July 2002 and September 2002.

In total 11 practitioners agreed to be interviewed by the principal investigator of the study. These included one medical officer and 10 medical assistants. Four questions were asked during the interview, these included the types of drugs they used for the treatment of brucellosis, diagnostic criteria for brucellosis, whether they have seen relapse or chronic brucellosis patients and their opinions on when most patients presented to hospital.

As hospitals visited did not have data in electronic form, files with data of the year 2001 were reviewed and all data recorded. These included data on the number of brucellosis cases, where they originated, when they were diagnosed and the treatment they received. The costs of consultation, drugs and investigations were also recorded.

All the data collected were entered and analysed on Microsoft Office Excel 2003 spreadsheets (Microsoft Corporation, Redmond, Washington, USA). Chi-square tests were used to analyze all data and Fisher's exact test was used where 2x2 tabular results were obtained with any expected counts of less than 5. A p-value of <0.05 was considered statistically significant. The average cost of treatment in the study was calculated from the sum of the total costs for consultation, diagnosis and treatment for all hospitals divided by the number of hospitals.

3.3   Results

3.3.1   Socio-demographic characteristics and distribution of brucellosis patients

Of the patients who presented to the hospitals in the study area between January 2001 and December 2001, 619 (0.36%) were diagnosed as suffering from brucellosis based on clinical history, clinical features, and a positive hospital serological test for brucellosis. For the purpose of this study all patients who were diagnosed and treated for brucellosis based on clinical features and positive hospital serology were named "brucellosis patients". Endulen hospital had the greatest proportion of brucellosis patients 1.8% (182/9923), followed by Dareda hospital 1.77 % (165/9297), and Karatu and Babati hospitals both with 0.4% (100/26963) and (40/8985) brucellosis patients respectively (Table 3.1). During the period of study, Katesh hospital did not have the antigen for testing brucellosis, so there was no available data on brucellosis from Katesh hospital.

Of brucellosis patients, 415 (67%) were female and 204 (33%) were male (χ^2 =22.17, df=4, P<0.01). Of these, 34 (5.4%) were 15 years of age and below, i.e. school age, 223 (36%) were 16-30 years of age, 138 (22.3%) were 31-45 years of age, 179 (29%) of age 46-60 and 45 (7.3%) more than 60 years of age (Table 3.2).

Table 3. 1: Brucellosis patients recorded from each of the study hospitals in the year 2001

Hospital	Number of patient	Males	Females	Total attendance	Percentage %
Karatu	100	25	75	26,963	0.40
Mbulu	46	17	29	14,944	0.30
Hydom	51	13	38	88,265	0.05
Katesh	-	-	-	-	-
Babati	40	8	32	8,985	0.40
Dareda	165	46	119	9,297	1.77
Wasso	35	8	27	11,968	0.30
Endulen	182	70	112	9,923	1.80
Overall	**619**	**204(30%)**	**415(70%)**	**170,345**	**0.36**

Table 3. 2: Age and sex distribution of brucellosis patients in the year 2001

Age group (years)	Number of patients			
	Males	Female	Total	Percentage (%)
=< 15	15	19	34	5.4
16 – 30	66	163	223	36.0
31 – 45	60	72	138	22.3
46 – 60	43	136	179	29.0
>60	20	25	45	7.3
Total	**204**	**415**	**619**	**100.0**

There was a significant difference in the number of seropositive patients identified between the two periods of the year i.e. the first and last quarters versus the middle two quarters of the year (χ^2= 13.1, df=6, p<0.05), with most brucellosis patients seen in the last quarter of the year (Table 3.3).

Table 3. 3: Distribution of brucellosis patients in the year 2001

Hospital	Jan – March	April – June	July – September	Oct. – December	Total
Karatu	29 (29%)	20 (20%)	23 (23%)	28 (28%)	100
Mbulu	7 (15%)	6 (13%)	18 (39%)	15 (33%)	46
Hydom	9 (18%)	13 (25%)	10 (20%)	19 (37%)	51
Babati	8 (20%)	12 (30%)	9 (23%)	11 (27%)	40
Dareda	42 (26%)	53 (32%)	30 (18%)	40 (24%)	165
Endulen	56 (31%)	23 (13%)	39 (21%)	64 (35%)	182
Wasso	8 (23%)	4 (11%)	9 (26%)	14 (40%)	35
Total (percentage)	159(25.7%)	131(21.2%)	138(22.3%)	191 (30.9%)	619

3.3.2 Knowledge of practitioners of clinical features of brucellosis and time of testing

Out of 11 clinicians interviewed, 10 thought brucellosis presents with non-specific clinical features that resemble malaria and typhoid such as headache, fever, joint pain and malaise, but they admitted to usually investigating for malaria and typhoid prior to brucellosis because they thought malaria and typhoid were much more prevalent. It was therefore not surprising to note that all practitioners thought brucellosis patients presented with clinical features of more than one month in duration.

3.3.3 Diagnostic criteria for brucellosis

All the practitioners interviewed mentioned the criteria for investigating and hence diagnosing brucellosis as a history of fever, joint pain, headache, general body malaise, fatigue and a positive blood test for brucellosis (see table 3.4 for the tests). By using the hospital tests (Table 3.4), the presence of clumps and the titre of 1:80 and above was used by the practitioners as positive tests for brucellosis.

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Different hospitals were found to use different antigens and techniques to test for brucellosis; some used a titration method while others used a plate or slide agglutination method. However antigens with the following brand names were seen in hospital laboratories, with Chronolab® containing *B. abortus* antigen being commonly used. Others included Merox® with *B. melitensis* and Biosystem® with *B. melitensis* and *B. abortus* antigens (Table 3.4)

Table 3. 4: Tests for brucellosis in different health facilities in Arusha and Manyara regions

Hospital	Test used/method	Antigen contained	Alternative antigen and method	Antigen contained
Karatu	Merox®, titration	*B. abortus* and *B. melintesis*	Rose Bengal (Nairobi), titration	*B. abortus*
Mbulu	Chronolab®, PA	*B. melitensis*	New market®, PA	*B. melitensis*
Hydom	New market®, titration	*B. melitensis*	Eurocell A®, titration	*B. abortus*
Katesh	Chronolab®, PA	*B. abortus*		
Dareda	Chronolab®, PA	*B. melitensis*	Rose Bengal (Nairobi) PA	*B. abortus*
Babati	Chronolab®, PA	*B. abortus*		
Wasso	Biosystem®, titration	*B. melitensis*	Eurocell A®, titration	*B. abortus*
Endulen	Chronolab®, PA	*B. abortus*	Humatex®, PA	*B. abortus*

PA – Plate/slide Agglutination
® - Brand names for antigens used in hospitals
Alternative antigens - Antigens used if the antigen of first choice from supplier is not available.

3.3.4 Therapeutic regimens used and their costs

In all the hospitals visited there was no clear policy for treating brucellosis patients. It was observed that within a single hospital practitioners used different regimens in

treating brucellosis patients. The different brucellosis treatment regimes, their costs and costs for treatment are summarized in Tables 3.5 and 3.6 respectively.

On average brucellosis patients in the study area used a total of 5,771[*] Tshs. for consultation, investigation and treatment of brucellosis (range 1,000-12,000 TShs.). Other indirect costs such as transportation costs, costs for accommodation and meals for patients and persons escorting them were not included.

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[*] The exchange rate was US $ 1 for 1880 TShs. in 2001

Table 3. 5: Therapeutic regimens used and costs charged by each hospital

Hospital	Drugs	Dosage/Duration	Cost
Karatu missionary hospital	1. Streptomycin injection	1 g once a day for two weeks	7,000 TShs.
	2. Doxycycline tablets	100 mg twice a day for four weeks	2,800 TShs.
	3. Cotrimoxazole tablets	960 mg twice a day for four weeks	2,800 TShs.
	4.Tetracycline tablets	500 mg four times a day for four weeks.	5,600 TShs.
Mbulu district Hospital	1. Doxycycline tablets	100 mg twice a day for four weeks	Cost for any treatment 1,000 TShs.
	2. Ceprofloxacin tablets	500 mg twice a day for two week	
	3.Streptomycin injection	1 g once a day for two weeks	
Haydom Lutheran hospital	1.Doxycycline tablets	100 mg once a day for four weeks	Cost for any treatment is 1,500 TShs.
	2.Tetracycline tablets	500 mg three times a day for two weeks	
	3. Cotrimoxazole tablets	960 mg twice a day for two weeks	
Hanang hospital	No antigen.		
Dareda hospital	1. Ciprofloxacin tablets	500 mg twice a day for ten days	Cost for diagnosis and treatment is 1,500 TShs.
	2. Doxycycline tablets	200 mg once a day for six weeks	
	3.Tetracycline tablets	200 mg once a day for six weeks	
Babati district Hospital	Erythromycin tablets	500 mg three times a day for three weeks	Cost for any treatment 1,000 TShs.
	2. Streptomycin injection	1 g once a day for three weeks	

Hospital	Drugs	Dosage/Duration	Cost
Wasso hospital	1. Streptomycin injection	1 g once a day for three weeks in combination with cotrimoxazole 960 mg twice a day for six weeks or doxycycline tablets 200 mg once a day for six weeks	Any kind of treatment offered the cost is 5,000 TShs.
Endulen hospital	1. Streptomycin injection	1g once a day for two weeks in combination with	Any kind of treatment offered the cost is 5,000 TShs.
	2. Doxycycline tablets	200 mg once a day for three weeks	
	If patient under 12 years of age	Cotrimoxazole alone for three to four weeks	

TShs. – Tanzanian shillings

Table 3. 6: Costs for diagnosis and treatment

Hospital	Service	Costs TShs.
Karatu	Consultation fee	500
	Investigation: Brucellosis	1,500
	Test for malaria	300
	Test for typhoid	1,500
	Treatment/drugs	4,900
	Total cost	**8,700**
Mbulu	Registration/ Consultation fee	300
	Investigation: Brucellosis	1,000
	Malaria	300
	Widal test	1,000
	Any treatment/drugs	1,000
	Total cost	**3,600**
Dareda	Cost for diagnosis and treatment	**1,500**
Hydom	Cost for diagnosis and treatment	**1,500**
Babati	Registration/ Consultation fee	300
	Investigation: Brucellosis	500
	Malaria	300
	Widal test	500
	Any treatment/drugs	1,000
	Total cost	**2,600**
Wasso hospital	Consultation	1,000
	Investigation: Brucellosis	2,000
	Malaria	500
	Typhoid	2,000
	Any treatment	5,000
	Total cost	**10,500**
Endulen hospital	Consultation	1,000
	Investigation: Brucellosis	3,000
	Malaria	Free
	Typhoid	3,000
	Any treatment	5,000
	Total cost	**12,000**

3.4   Discussion and conclusion

More women were diagnosed as suffering from brucellosis than males in the year 2001, the difference was statistically significant ($p<0.05$). It is possible that women who engage more in farm and home-based activities such as looking after animals, milking and cleaning pose themselves to a greater risk of brucellosis than men. Studies conducted in Spain and Egypt by Troy *et al.*, (2005) and Hussein *et al.*, (2005) respectively also showed similar results that brucellosis is much more common in women than men. In the current study most of the brucellosis patients were 16-30 years of age and fewer were at pre-school age, school-age or in old-age. In the study area most of the individuals of 16-30 years of age are married and are actively involved in different home or farm-based activities. These results suggest that such activities might pose a greater risk to them than to other age groups that are less involved with such activities.

More brucellosis patients were recorded in the beginning and towards the end of the calendar year at a time when most parts of the study area had slight rains, farmers were either preparing the land for planting or had already planted their crops. It is possible at this time of the year there was more transmission of brucellosis or it is possible that farmers had sold their crops and were better able to afford the costs of treatment. More studies need to be conducted to establish the reasons for this seasonal distribution of brucellosis patients.

The majority of practitioners thought brucellosis patients presented with non-specific clinical features that resemble malaria and typhoid, but it was a common practice for

them to investigate for malaria and typhoid first because they thought malaria and typhoid were much more prevalent than brucellosis. The practice of not testing routinely for brucellosis is likely to cause a delay in diagnosis and treatment of brucellosis.

All the practitioners interviewed mentioned the criteria for investigating and hence diagnosing brucellosis as a history of fever, joint pain, headache, general body malaise, fatigue and a positive test for brucellosis. This agrees with literature (Onyemelukwe, 1989; Sadat-Ali et al., 1991; Al-Shamahy and Wright, 2001; Tsolia et al., 2002; Chadda et al., 2004; Fallatah et al., 2005; Mantur et al., 2004; Galukande et al., 2005 ; Andriopoulos et al., 2006 and Kokoglu et al., 2006) showing that practitioners generally had the correct knowledge on the presentation and diagnosis of brucellosis.

Different hospitals were found to use different antigens and techniques to test for brucellosis such as Chronolab® containing B. abortus, Merox® with B. melitensis and Biosystem® with B. melitensis and B. abortus antigens. As the sensitivities and specificities of these antigenic tests are not known, there is a possibility that brucellosis is misdiagnosed in the area.

It was evident that no standardized treatment protocol for brucellosis existed in the study districts. Even drugs that are used for the treatment of tuberculosis such as streptomycin and rifampicin were found to be used at different doses and for different durations. It was also observed that due to a short supply of drugs, practitioners

prescribed different types of drugs, some in combination and some alone at different doses, depending on their availability. This practice could predispose to the emergence of drug resistant strains not only of *Brucella* but also tuberculosis and other bacterial pathogens (Al-Hajjaj *et al.,* 2001; Al Dahouk *et al.,* 2005; Bayindir *et al.,* 2003). According to the Ministry of Health, the standard treatment guideline and the National Essential Drugs List for Tanzania (NEDLIT) produced in 1998, requires that a combination of either doxycycline or tetracycline for four weeks and streptomycin injection for the first two weeks should be used for the treatment of brucellosis. This was not practiced in the study area.

Brucellosis patients used an average of 5771 TShs. for consultation, investigation and treatment of brucellosis. This excludes the costs of transportation to and from the hospital and the costs of family members escorting them (see chapter 8). It is possible that the majority of farmers who depend on subsistence farming for their livelihood would not afford if several members of a household were to suffer from brucellosis in a year. Apart from morbidity, brucellosis is therefore likely to have a significant socio-economic impact on the poor farmers in the study area.

There is an urgent need for evaluation of brucellosis treatment regimes and diagnostic tools in Tanzania with an aim of establishing and hence adopting standardized treatment and diagnostic protocols. This would improve the diagnosis and treatment outcome and prevent emergence of drug resistant strains of *Brucella, Mycobacteria* and other bacterial pathogens. The government and hospitals should ensure that the recommended drugs and reagents for the tests are available throughout the year.

There is a need to carry out a study that would establish to what extent brucellosis is a problem, not only to the hospital-going population, but also in the communities in Arusha and Manyara regions. Other areas into which further research is required include risk factors for brucellosis, health seeking behavior and clinical features of brucellosis patients.

4. Cross-sectional study of human brucellosis in rural communities in northern Tanzania

4.1   Summary

Objectives: To determine the seroprevalence of human brucellosis in Arusha and Manyara regions. The study also aimed to determine the relationship between infection in humans and in livestock and the relationship between the serostatus of human brucellosis and clinical manifestations of the disease.

Methodology: A cross-sectional survey was conducted between May 2002 and July 2003 in the districts of Karatu and Ngorongoro in Arusha region and Mbulu, Babati and Hanang in Manyara region. Villages, tencell units and households for blood sampling were obtained by multistage cluster sampling. Samples of blood collected were tested for brucellosis using the RBPT in the field and at the SUA and using the c-ELISA test at the VLA. For all the RBPT positives, clinical features were recorded. Open and closed ended questionnaires to establish the knowledge, attitude and practice of the people towards brucellosis and other zoonotic diseases were also administered.

Results: Based on the RBPT results, the seroprevalence of brucellosis was 4.8% in the field and 6.4 % at the SUA, and using the c-ELISA test conducted at the VLA which was the gold standard test the seroprevalence of brucellosis was 7.7%. The majority of Rose-Bengal positive individuals (56.5%) were asymptomatic. Of those with clinical features, fever, joint pain and backache were the most common. There was a significant association between prevalence of brucellosis in humans and the prevalence of brucellosis in goats at district level ($p < 0.05$).

Discussion and conclusion: There could be a considerable number of brucellosis cases in Arusha and Manyara regions that do not present to hospital due to various reasons. Estimation of the burden caused by brucellosis should also take into account members of the community that meet the diagnosis criteria. Community members should be made aware of the danger posed by goats in transmitting brucellosis particularly during handling fluids

from goats such as blood and during assisting parturition. Care should be taken in choosing the type of serological test to be performed in prevalence studies as some tests might not give the true brucellosis prevalence.

4.2 Introduction

Zoonotic diseases have a major impact on the health and economic prosperity of the developing world (Smits and Cutler, 2004). Brucellosis is one of the most important bacterial zoonoses worldwide caused by bacteria of the genus *Brucella* (Young, 1995a). *Brucella* is responsible for enormous economic losses as well as considerable human morbidity and mortality in endemic areas (Boschiroli *et al.*, 2001). The losses are much more evident in developing countries where disease control strategies do not exist, or have collapsed. Poor farming systems have also contributed to difficulties in the control of animal movement and disease monitoring (Schelling *et al.*, 2003; McDermot and Arimi, 2002).

Humans contract brucellosis mainly from domestic animals including cattle, sheep and dogs, but further complications arise through wild animal reservoirs which may also carry and transmit the disease (Godfroid, 2002). Transmission from infected livestock to man can either be direct through contact with infected material, or indirect through consumption of animal produce. In man, the disease may affect almost any organ such as musculo-skeletal organs, the heart, lungs, liver, brain, spleen and kidneys, which, if not treated early may lead to severe and prolonged disability (Corbel, 1997). *B. melitensis* is the most common species infecting man and is probably the most virulent, followed by *B. suis* and *B. abortus* (Domenech *et al.*, 1983). Brucellosis caused by *B. melitensis* in humans is therefore more prolonged and more severe and debilitating than brucellosis caused by *B. abortus* or *B. suis* (Domenech *et al.*, 1983). In animals *B. melitensis* infection occurs more commonly in small ruminants such goats and sheep whereas *B. abortus* occurs more commonly in cattle (Corbel *et al.*, 1984; Crawford *et al.*, 1990).

Brucellosis remains a major problem in many parts of the world, notably in the Mediterranean region, western Asia, and parts of Africa and Latin America (Corbel, 1997). Even in some countries of Western Europe where it has been eradicated, there is still a constant threat of reintroduction via livestock trading (Whatmore et al., 2005). In a cross-sectional study that was conducted in two provinces of Anatolia, Turkey, the seroprevalence of brucellosis in humans ranged from 2.9 to 8.5% using the Rose Bengal test. Adherence to traditional farming practices and lifestyle and a preference for fresh dairy products were found to contribute to the high seroprevalence of brucellosis in the two provinces (Kose et al., 2006). In a random survey conducted in Punjab (India), the overall prevalence of brucellosis in humans using ELISA was 11.23% (Dhand et al., 2005).

Brucellosis has been reported from almost all countries in Africa (Refai, 2002). It is prevalent in all major livestock production systems in sub-Saharan Africa, yet it is often unrecognised through lack of awareness by both veterinarians and human health staff and the absence of accessible laboratory diagnostic facilities (McDermott and Arimi, 2002). This has resulted in the disease being largely neglected with little control and prevention in many parts of the continent (McDermott and Arimi, 2002). Worldwide, the incidence of brucellosis is highest in pastoral production systems where large numbers of animals mix, and is lowest for confined farms. This has made the control of brucellosis more problematic in pastoral or migratory populations, which in Africa is practiced by a significant proportion of the agricultural population (Schelling et al., 2003).

Although brucellosis was first confirmed in Tanzania in 1928 (Kitaly, 1984), very few studies on brucellosis have focused on the burden of the disease in the traditional sector that makes up more than 97% of the national total herd. The majority of the studies have been conducted in dairy farms and ranches (Mahlau, 1967; Kitaly, 1984; Minga and Balemba, 1990; Jiwa *et al.*, 1996; Weinhaulp *et al.*, 2000), where the disease was suspected, and only a few studies used random methodology to sample the study population. In addition, studies on brucellosis have mainly been carried out by veterinary professionals. In humans, most of community-based studies conducted in Tanzania have focused on malaria (Omombu *et al.*, 2004; Bodker *et al.*, 2006; Wang *et al.*, 2006; Kachur *et al.*, 2006) and HIV (Matee *et al.*, 2006; Somi *et al.*, 2006; Nielsen *et al.*, 2006; Kwesigabo *et al.*, 2005), which have been regarded as of greater public health importance. In Tanzania many zoonotic diseases such as brucellosis have generally been regarded as not posing a serious threat to human health. It is not surprising therefore to see that to date, no studies have been conducted to determine the distribution and characteristics of human brucellosis in Tanzania.

The current study aims to determine the seroprevalence of human brucellosis in Arusha and Manyara regions and to examine the relationship between brucellosis seropositivity and farming systems. The study also aims to determine the relationship between brucellosis in humans and in livestock and to examine the relationship between serostatus and clinical manifestation of brucellosis.

4.3 Materials and methods

4.3.1   Study area and methodology

The study area and the methodology involved have been described in detail in the second chapter of this work. A brief account of the methodology is described.

The study was conducted between May 2002 and July 2003. In the household cross-sectional survey a multistage cluster sampling was conducted from the village level, the ten-cell leader unit and finally to the household level. Random tables were used in the random selection. The sampling frame comprised all of the 285 villages in the study area. From the sample, 32 villages comprising 20 pastoral and 12 agropastoral villages were randomly selected.

At the household level all members of the households were encouraged to be tested for brucellosis and for livestock the number of each species to be sampled was determined according to the sample size needed to detect infection in a herd, using 99% confidence and power of 80% and prevalence of 5% (Thrusfield, 2001). Questionnaire data collection on the knowledge, attitude and practice of the people towards brucellosis and other zoonoses was then undertaken. Respondents were members of households and information was obtained by posing a question after which household members gave responses after discussion. In some households, translators had to be used to ease communication between the investigator and respondents.

Human and livestock blood sampling and testing with the RBPT was conducted in the field (see section 2.4.5) and feedback of human results conveyed to those tested, and those of livestock

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conveyed to the head of the household on the same day. In both cases, confidentiality was observed. Serum samples were stored for the RBPT and the c-ELISA test at the SUA and the c-ELISA test at the VLA.

For all the RBPT positive individuals, history of any symptoms was recorded and physical examination for any clinical features of a disease was undertaken. For the c-ELISA positive individuals it was not possible to record their clinical picture as samples had to be taken to the VLA, UK for testing and there was a delay in getting the feedback of results. During storage and shipment, some samples leaked and some were lost. This explains the differences in the number of samples tested in the field, at the SUA and at the VLA.

Plate 4. 1: Administering questionnaires in the cross-sectional study

Plate 4. 2: The RBPT in the field

4.3.2   Data analysis

All data were entered on Microsoft Office Excel 2003 spreadsheets (Microsoft Corporation,

Redmond, Washington, USA). Using the VLA c-ELISA result as a binary response variable,

logistic regression analysis was conducted to analyse the association between brucellosis

seropositivity and sex, age and farming systems. Test statistics with a p-value of <0.05 were

considered significant. In the analysis of the relationship between the prevalence of brucellosis in

humans and in livestock at the household, the village and the district levels, the prevalence of

brucellosis was calculated from the total number of c-ELISA positives divided by the total

number of all those tested using the VLA c-ELISA test at that particular level and the result was

multiplied by 100 to obtain a percentage.

The prevalence of brucellosis in the study area was calculated from the total number of all

seropositives (by the RBPT or by the c-ELISA test) divided by the total number of all tested in

the fourteen months period. All the prevalence values were converted to percentages by

multiplying by 100.

4.4 Results

4.4.1   Individuals tested

A total of 106 randomly selected households in 29 villages were visited in Karatu, Mbulu, Hanang, Babati and Ngorongoro districts between May 2002 and July 2003. Of these, 12 households were from Babati, 14 from Hanang, 38 from Karatu, 18 from Mbulu and 24 from Ngorongoro districts. Fourteen household leaders did not consent to participate with the study activities, hence other households were selected randomly as replacements. In total, 476 individuals from the above households were tested for brucellosis (Table 4.1). Of those tested, 249 (50.4%) were male and 236 (49.6%) were female (Table 4.2).

Table 4. 1: Villages visited and individuals tested

District	Village	Number of individuals tested
Babati	Bermi	14
	Mamire	7
	Singe	35
Hanang	Getasam	26
	Masqaroda	12
	Measkroni	8
	Mulbadaw	4
Karatu	Ayalalio	15
	Basodawish	35
	Chemchem	19
	Endamaghan	1
	Getamock	14
	Gyekrum Lambo	16
	Kambi ya Simba	15
	Kilima moja	25
	Qanded	3
	Qurus	7
	Rhotia kati	37
Mbulu	Dirim	22
	Endagikoti	21
	Garbabi	22
	Murray	10
	Sanu Baray	18
Ngorongoro	Enduleni	23
	Esere	17
	Kakesio	9
	Malambo	24
	Oloipiri	6
	Soitsambu	11
	Total	**476**

Table 4. 2: Sex distribution of individuals tested for brucellosis

Sex	District Babati	Hanang	Karatu	Mbulu	Ngorongoro	Total
Female	28 (50%)	23 (46%)	89 (48%)	45 (48%)	51 (57%)	236 (49%)
Male	28 (50%)	27 (54%)	98 (52%)	48 (52%)	39 (43%)	240 (51%)
Total	**56**	**50**	**187**	**93**	**90**	**476**

4.4.2 Results of the field RBPT

Of the 476 individuals tested for brucellosis using the RBPT in the field, 23 (4.8%) were

seropositive. Fifteen of these (8.0%) were from Karatu district, four (4.4%) from Ngorongoro,

two (4.0%) were from Hanang, and two (4.4%) were from Mbulu. None of the individual tested

in Babati district gave a positive result (Table 4.3).

Table 4. 3: Results of the field RBPT

Field RBPT	District Babati	Hanang	Karatu	Mbulu	Ngorongoro	Total
Negative	56(100%)	48(96%)	172(92.0%)	91(97.9%)	86(95.6%)	453(95.2%)
Positive	0 (0%)	2(4%)	15(8.0%)	2(2.1%)	4(4.4%)	23(4.8%)
Total	**56**	**50**	**187**	**93**	**90**	**476**

4.4.3 Results of the RPBT and the c-ELISA test conducted at the SUA laboratory

At the SUA laboratory, only 280 samples were tested using the c-ELISA test. Of these, 18 (6.4

%) tested positive. The RBPT was performed on 427 samples and of these 3.7% tested positive

for brucellosis (Table 4.4)

Table 4. 4: Results of the c-ELISA test and the RBPT at the SUA laboratory

c-ELISA	District					
	Babati	Hanang	Karatu	Mbulu	Ngorongoro	Total
Negative	48 (100%)	47(95.9%)	167(91.3)	0(0%)	0(0%)	262(93.6%)
Positive	0(0%)	2(4.1%)	16(8.7%)	0(0%)	0(0%)	18(6.4%)
Total	48	49	183	0	0	280
Laboratory RBPT						
Negative	47(97.9%)	49(98%)	174(95.1%)	69(97.2%)	72(96%)	411(96.3%)
Positive	1(2.1%)	1(2%)	9(4.9%)	2(2.8%)	3(4%)	16(3.7%)
Total	48	50	183	71	75	427

4.4.4 Results of the c-ELISA test at the VLA

Thirty three samples (7.7%) of 395 human samples collected in the fourteen month period tested positive for the c-ELISA test at the VLA. Ngorongoro district recorded the highest proportion of seropositives. None of the samples from Babati was seropositive (Table 4.5). The majority of seropositive individuals belonged to the age group 16-30 years of age (Table 4.6). The locations of the c-ELISA positives and negatives are shown in Figure 4.1.

Table 4. 5: Results of the c-ELISA test at the VLA

c-ELISA results	Babati	Hanang	Karatu	Mbulu	Ngorongoro	Total
Negative	56(100%)	44(95.7%)	155(88.6%)	88(96.8%)	52(86.7%)	395(92.3%)
Positive	0(0%)	2(4.3%)	20(11.4%)	3(3.2%)	8(13.3%)	33(7.7%)
Total	56	46	175	91	60	428

Figure 4. 1: Map of Arusha and Manyara regions showing the locations of households of VLA c-ELISA negative and positive individuals
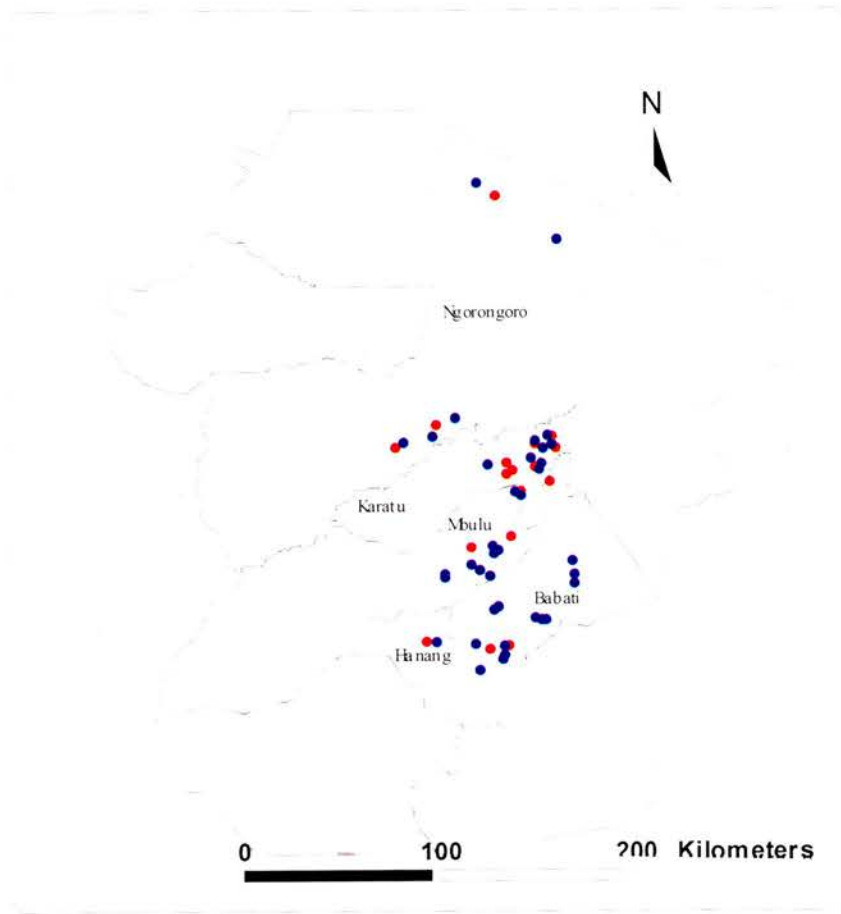
Key

- Household with seropositive individual/s
- Household of seronegative individual/s

Table 4. 6: Age and sex distribution of individuals who tested positive with the c-ELISA test conducted at the VLA.

Age groups	Female	Male	Total	Percentage
1-15	2	1	3	9.09
16-30	8	7	15	45.45
31-45	4	5	9	27.27
46-60	2	2	4	12.12
>60	1	1	2	6.06
Total	**17**	**16**	**33**	**100.00**

4.4.5   Brucellosis in pastoral and agropastoral areas

Of the 395 individuals who were tested with the c-ELISA test at the VLA, 15.4% (8/52) were

seropositive in pastoral areas and 7.3% (25/343) were seropositive in agropastoral areas (Table

4.7).

Table 4. 7: Brucellosis in pastoral and agropastoral areas

District	Predominant farming system	VLA c-ELISA results	
		Positive	**Negative**
Babati	Agropastoral	0 (0 %)	56
Karatu	Agropastoral	20 (11.4%)	155
Mbulu	Agropastoral	3 (3.2%)	88
Hanang	Agropastoral	2 (4.3%)	44
Total in agropastoral		**25 (7.3%)**	**343**
Ngorongoro	Pastoral	8 (15.4%)	52
Total in pastoral		**8 (15.4%)**	**52**

4.4.6 Test of association between brucellosis seropositivity and sex, age and farming system.

Using the VLA c-ELISA results as a binary response variable, a logistic regression

analysis was conducted to test for association between brucellosis seropositivity and sex,

age and farming system. Results showed that there was no significant association

between brucellosis seropositivity and age, sex and farming system (Table 4.9). When

age was aggregated into two age groups with children 15 years and less and adults with

greater than 15 years of age, there was a significant number of those with greater than 15

years (Table 4.8).

Table 4. 8: Analysis for association between brucellosis seropositivity and sex, age and farming system

Variable	Coefficients	p-value	OR	95% confidence interval	
				Upper	Lower
Age (Continuous)	0.00	0.90	1.00	0.98	1.02
Sex	0.15	0.65	1.17	0.60	2.26
Farming system	0.01	0.77	1.01	0.49	2.11
Age (cut-off point 15 years)	0.12	0.001	3.4	1.23	6.24

4.4.7 Clinical features observed in the RBPT and the c-ELISA positive individuals

Of the 23 individuals who tested positive with the RBPT during the cross-sectional survey, only 10 (43.5%) had clinical features suggestive of brucellosis. The most common clinical features in the RBPT positive individuals were fever, joint pain, backache, headache and general body malaise. Of those who tested positive with the RBPT, 10 (43.5%) individuals also tested positive with the c-ELISA test performed at the VLA. Out of these, five (50%) had clinical features suggestive of brucellosis (Table 4.9).

Table 4. 9: Clinical features observed in the RBPT and the c-ELISA positive individuals

Symptom	RBPT positives with symptoms	Percentage RBPT positives with symptoms	c-ELISA positives with symptoms	Percentage c-ELISA positives with symptoms
Fever	9	39.0	4	40
Joint pain	8	34.0	4	40
Backache	6	26.0	2	20
Headache	5	22.0	4	40
General body malaise	3	13.0		
Chest pain	2	9.0	2	20
General body weakness	2	9.0		
Loss of appetite	1	4.5		
Cough	1	4.5		
Adenitis	1	4.5		
Abdominal discomfort	1	4.5	1	10

4.4.8   The relationship between the prevalence of brucellosis in livestock and in humans at household, village and districts levels

The relationship between prevalence of brucellosis in livestock and in humans was analysed at household, village and district levels. VLA c-ELISA results were used in the analysis. The following is a summary of statistics for regression analysis carried out at different levels.

4.4.8.1 Relationship between the prevalence of brucellosis in livestock and in humans at the household level

At the household level there was no significant association between the prevalence of brucellosis in livestock and in humans (F-statistic=0.03, df=1, p-value =0.87) (Figure 4.2).

Figure 4. 2: Relationship between the prevalence of brucellosis in livestock and in humans at the household level.
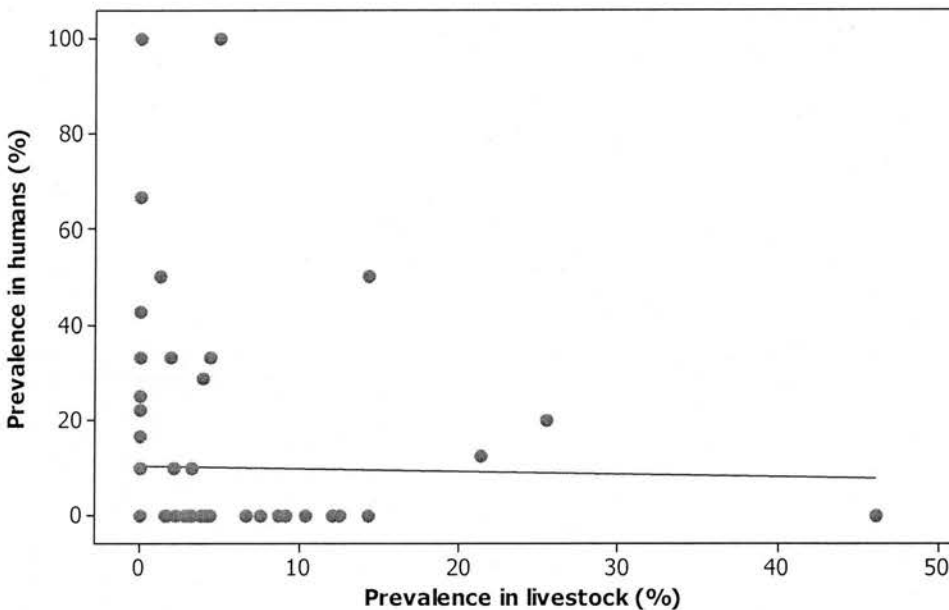
4.4.8.2 Relationship between the prevalence of brucellosis in livestock and in humans at the village level

At the village level there was no significant association between the prevalence of brucellosis in livestock and in humans (F-statistic=1.09, df=1, p-value = 0.31) (Figure 4.3).

Figure 4. 3: Relationship between the prevalence of brucellosis in livestock and in humans at the village level
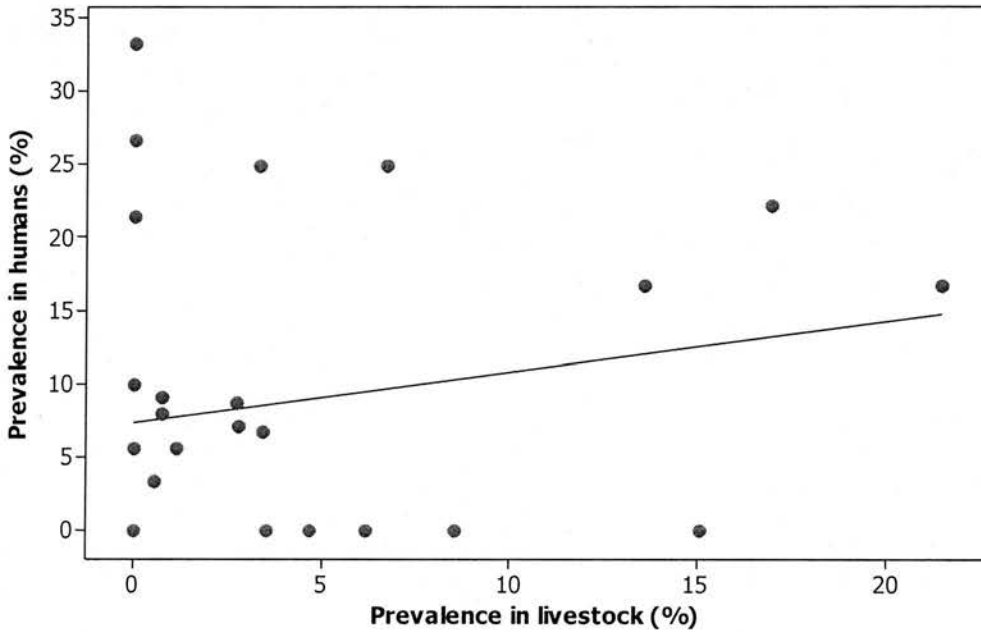
4.4.8.3 Relationship between the prevalence of brucellosis in cattle and in humans at the village level

At the village level there was no significant association between the prevalence of brucellosis in cattle and in humans (F-statistic=0.28, df=1, p-value=0.6) (Figure 4.4).

Figure 4. 4: Relationship between the prevalence of brucellosis in cattle and in humans at the village level
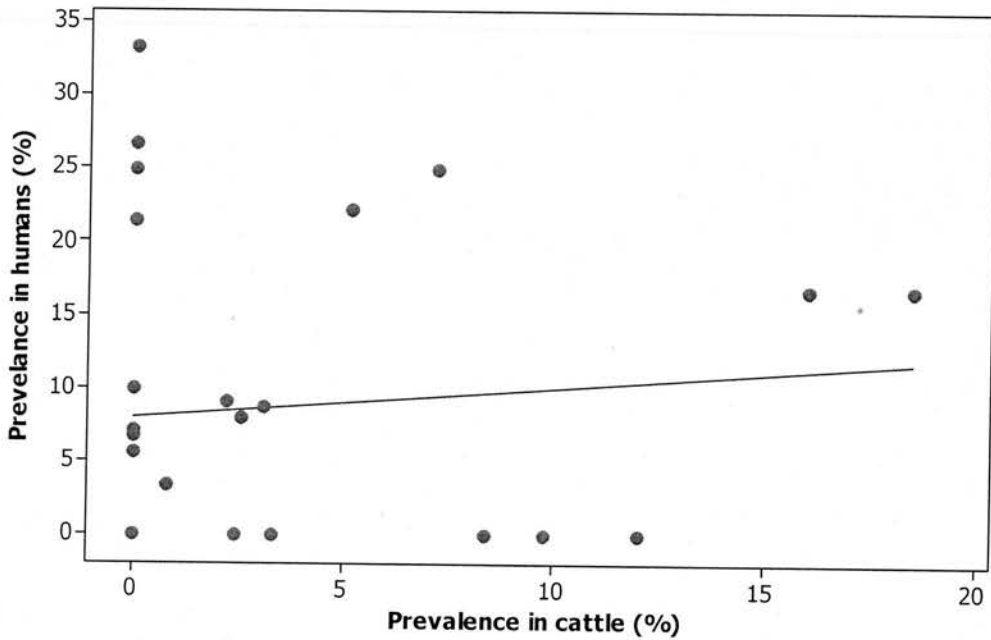
4.4.8.4 Relationship between the prevalence of brucellosis in humans and in goats at the village level

There was no significant associaton between the prevalence of brucellosis in goats and the prevalence of brucellosis in humans at the village level (F-statistic=1.36, df=1, p-value=0.25) (Figure 4.5).

Figure 4. 5: Relationship between the prevalence of brucellosis in goats and in humans at the village level
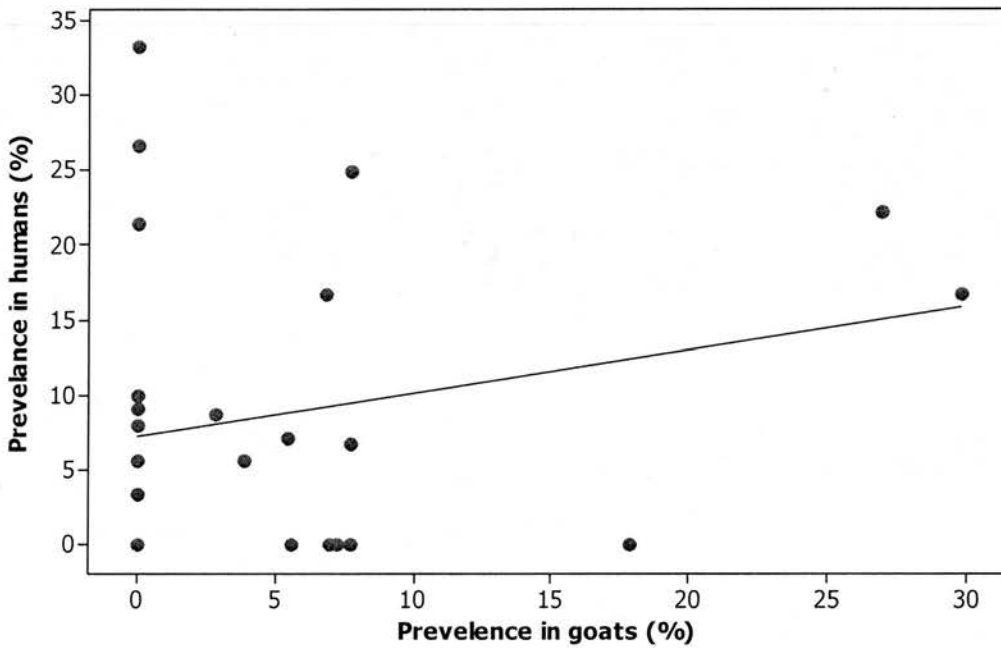
4.4.8.5 Relationship between the prevalence of brucellosis in sheep and the prevalence of brucellosis in humans at the village level

At the village level there was no significant association between the prevalence of brucellosis in sheep and the prevalence of brucellosis in humans (F-statistic=0.02, df=1, p-value=0.88) (Figure 4.6).

Figure 4. 6: Relationship between the prevalence of brucellosis in sheep and the prevalence of brucellosis in humans at the village level
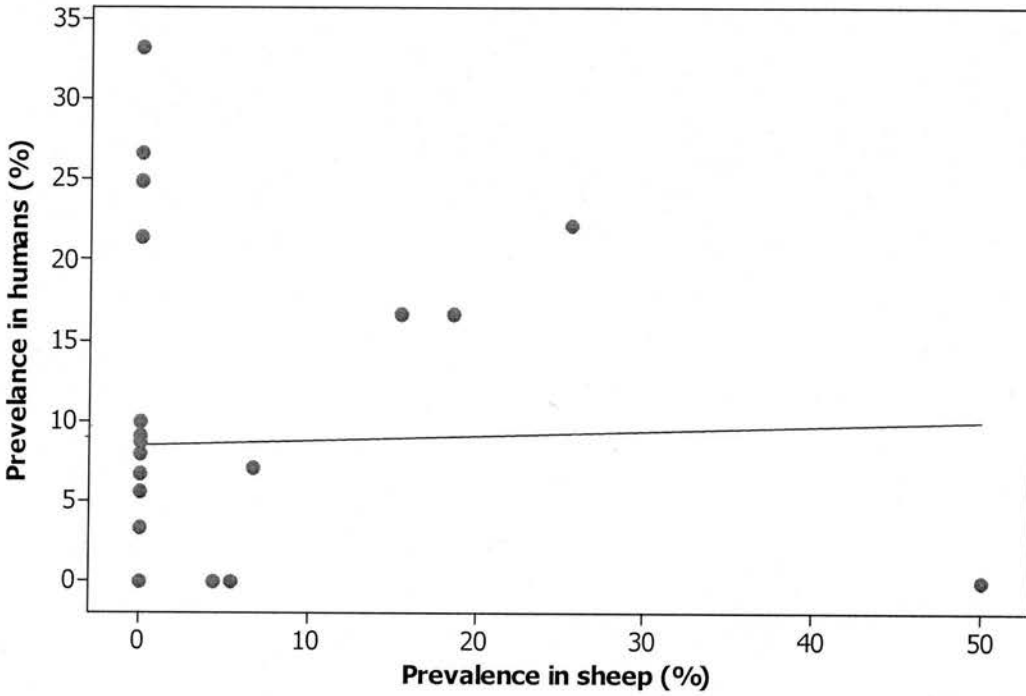
4.4.8.6 Relationship between the prevalence of brucellosis in livestock and prevalence of brucellosis in humans at the district level

At the district level there was no significant association between the prevalence of

brucellosis in livestock and in humans (F-statistic=0.09, df=1, p-value=0.78) (Figure 4.7).

Figure 4. 7: Relationship between the prevalence of brucellosis in livestock and in humans at district level
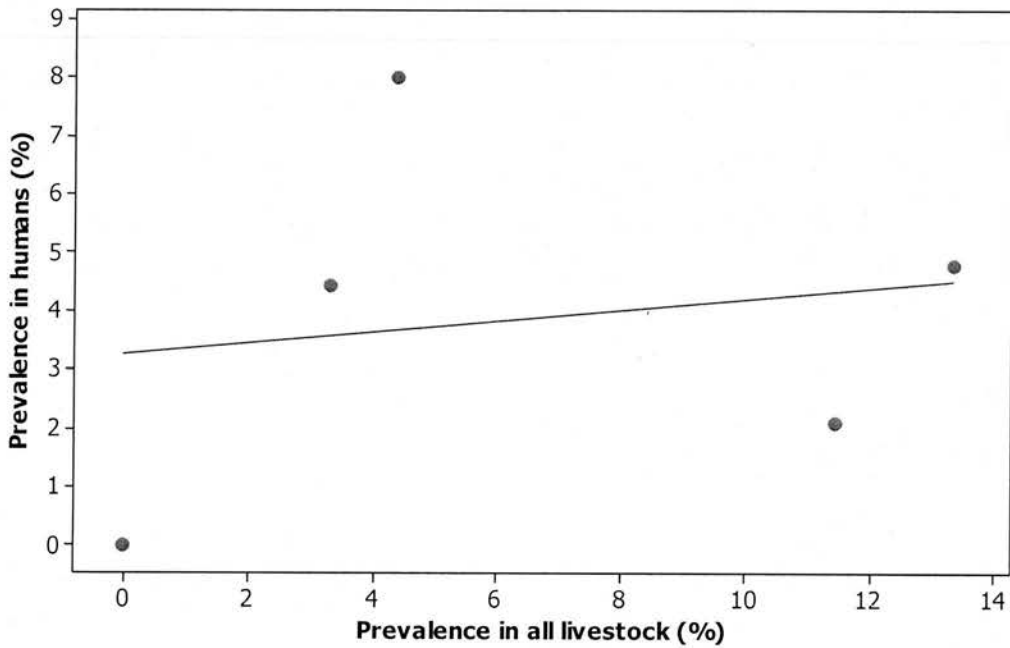
4.4.8.7 Relationship between the prevalence of brucellosis in cattle and the prevalence of brucellosis in humans at the district level

There was no significant association between the prevalence of brucellosis in cattle and in humans at the district level (F-statistic=4.89, df=1, p-value=0.11) (Figure 4.8).

Figure 4. 8: Relationship between the prevalence of brucellosis in cattle and in humans at the district level
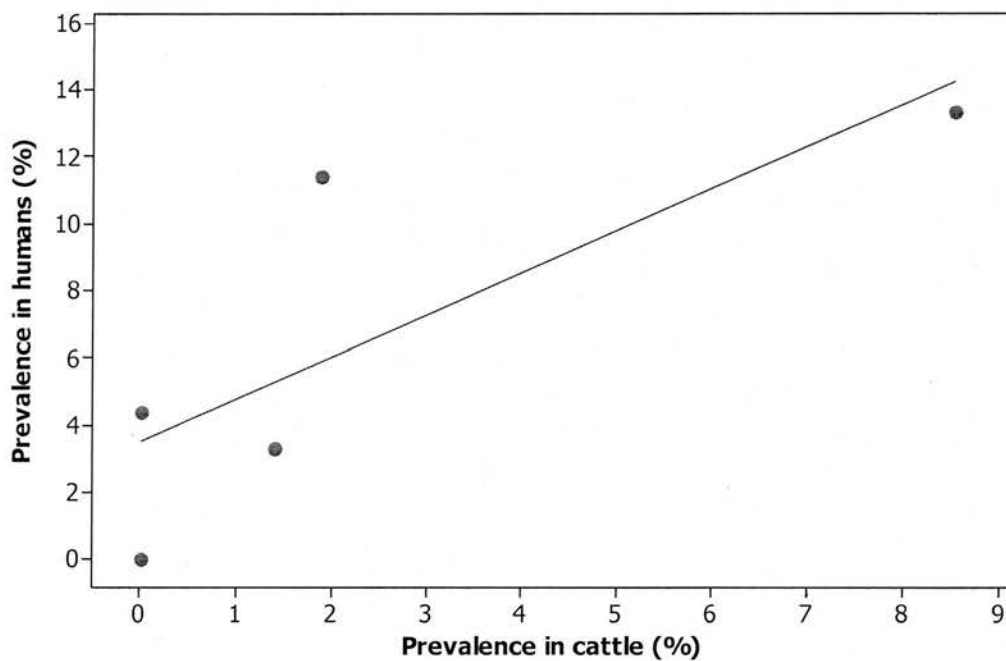
4.4.8.8 Relationship between the prevalence of brucellosis in goats and in humans at the district level

At the district level there was a significant association between the prevalence of brucellosis in goats and the prevalence of brucellosis in humans (F-statistic=11.44, df=1, p-value=0.04) (Figure 4.9).

Figure 4. 9: Relationship between the prevalence of brucellosis in goats and the prevalence of brucellosis in humans at the district level
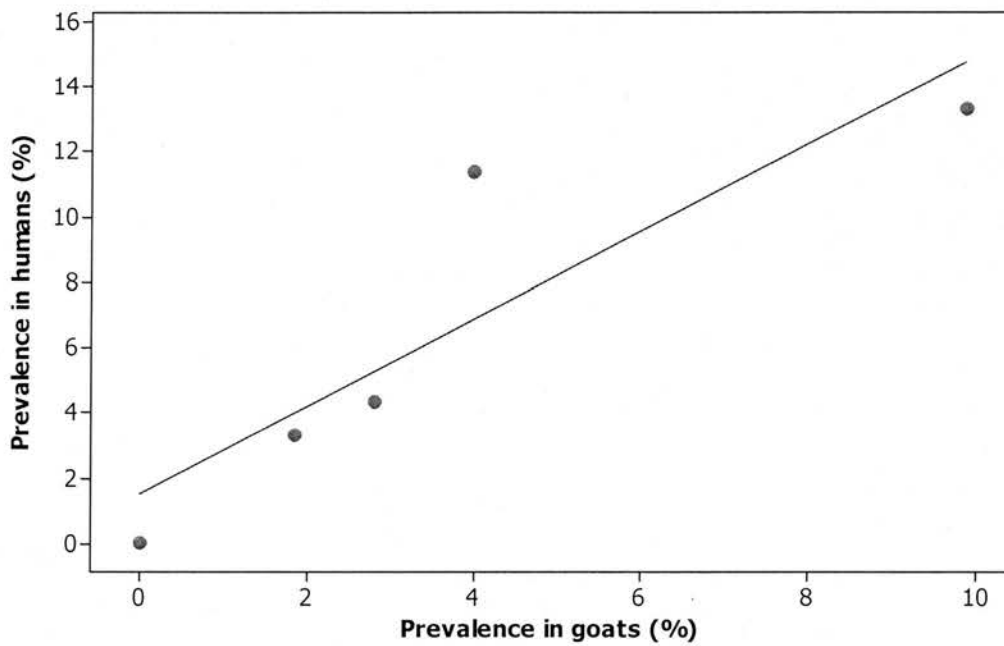
4.4.8.9 Relationship between the prevalence of brucellosis in sheep and the prevalence of brucellosis in humans at the district level

At the district level the association between prevalence of brucellosis in sheep and the

prevalence of brucellosis in humans was not statistically significant (F-statistic=6.53, df=1,

p-value=0.08) (Figure 4.10).

Figure 4. 10: Relationship between the prevalence of brucellosis in sheep and the prevalence of brucellosis in humans at the district level
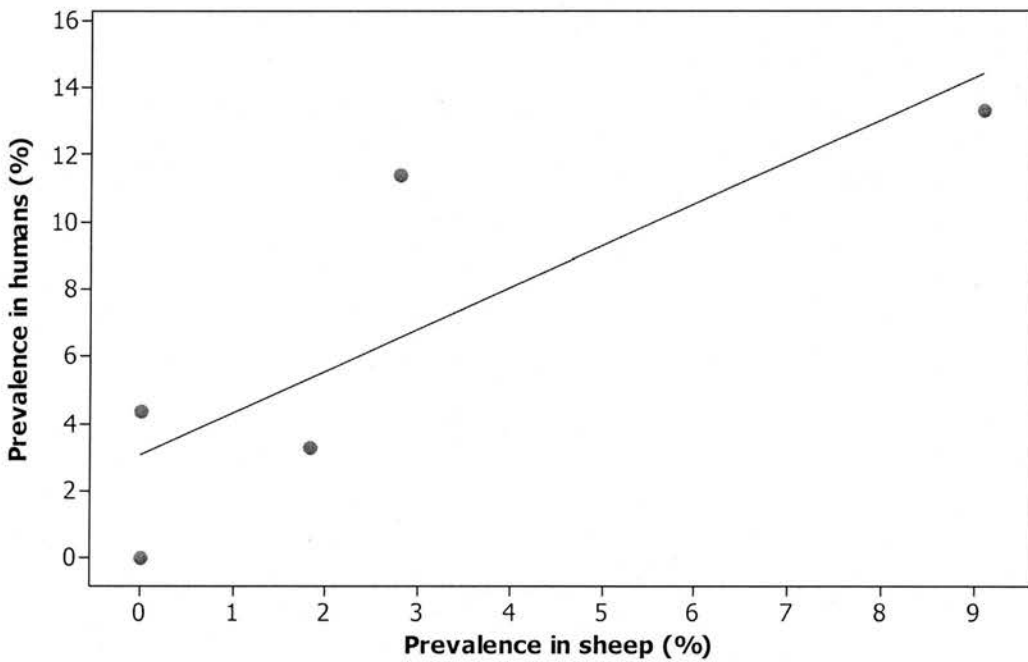
4.5 Discussion

Seropositivity was found to be higher in individuals of above 15 years of age than those below 15 years of age. The results were similar to those obtained in Yemen by Al-Shamahy and Wright (2001), in which seropositivity was found to be more common in adults than in children. The cut-off point of 15 years was used to distinguish between school children and adults who are fully involved with home and farm based activities. Seropositivity was higher in the age group 16-30 years, and was low in the very elderly (>60 years). Younger age groups carry more YLL and YLD than the aged who are approaching the end of their life span (see section 8.3.3). Since DALYs is the sum of YLL and YLD (Murray and Lopez, 1994), the age pattern of brucellosis cases is likely to give higher DALY. As most of the cases are in the productive age, the burden caused by brucellosis is likely to have also an impact on the economies of the households of the affected and Tanzania as a whole.

Of the 23 individuals who tested positive with the RBPT in the field, only 10 (43.4%) individuals had clinical features suggestive of brucellosis. Of these, five (21.7%) individuals were c-ELISA positive and had symptoms suggestive of brucellosis. Most of the asymptomatic c- ELISA positive individuals were likely to be in the subclinical stage or were in the incubation period. The majority of the individuals in subclinical stage or incubation period eventually develop the acute clinical disease. It is also possible that some of the RBPT positives individuals were not actually due to *Brucella* but rather due to cross-reaction with other bacteria such as *Yersinia* or *Salmonella* (Staak *et al.*, 2000; Gourdon, 1999) which cause mild or no clinical disease in humans.

The most common clinical features in seropositive individuals were fever followed by joint pain and backache. Similar findings were obtained in other studies (Tsolia *et al.*, 2002; Mantur *et al.*, 2004; Fallatah *et al.*, 2005; Kokoglu *et al.*, 2006) where fever and joint pain were cited as the most common clinical feature of brucellosis. Others include back pain (Sadat-Ali *et al.*, 1991; Chadda *et al.*, 2004; Galukande *et al.*, 2005), malaise, headache, drenching sweat, spleen and liver enlargement (Onyemelukwe, 1989; Al-Shamahy and Wright, 2001; Andriopoulos *et al.*, 2006). However, additional trace-backs and thorough physical examination of the c-ELISA positives are required to provide a better clinical picture of brucellosis cases in the study area.

The c-ELISA test and the RBPT were used in different settings to provide information on the prevalence of brucellosis. Based on the RBPT, the seroprevalence of brucellosis was 4.8% in the field, and 6.4 % at the SUA and based on the c-ELISA test performed at the SUA and the VLA, the seroprevalences of brucellosis were 3.7% and 7.7% respectively. The differences in the results may be attributed to the differences in the sensitivities and specificities of the tests and the personnel involved in testing. The c-ELISA test conducted at the VLA was the gold standard test in the study. The seroprevalence of brucellosis based on the VLA c-ELISA results was therefore taken as the true seroprevalence and was compared with other seroprevalence results. All other tests showed lower seroprevalence compared to the VLA c-ELISA seroprevalence results. The discrepancies in the results obtained indicated that care should be taken when choosing the type of test to be used for prevalence studies as some tests might not give the true picture of the disease. It is important that a test is evaluated first before using it in prevalence studies. Analysis and discussion on brucellosis diagnostic tests are described in chapter 7 of this thesis.

The prevalence of brucellosis in humans did not show a significant association with the prevalence of livestock at the household level. This finding agrees with what was established during the case-control study (see chapter 6), in which no association was established between brucellosis cases and their own livestock. There was a significant association between the prevalence of brucellosis in humans and prevalence of brucellosis in goats at the district level. Goats within the district were therefore more strongly associated with brucellosis than cows and sheep at household and village levels. In the univariate analysis of risk factors for brucellosis (see chapter 6), brucellosis was found to be significantly associated with a household where a goat had aborted. Since milk from goats is not widely consumed in the study area, the transmission of brucellosis in the study area is most likely by contact with infected goats during assisting parturition. The finding could also be an indication that *B. melitensis* which is most commonly found in small ruminants such as goats and sheep is the main cause of brucellosis in the study area. Although in a concurrent study conducted by Shirima *et al.*, (2005) in Arusha and Manyara regions, *B. melitensis* was isolated, a large number of samples did not show any growth due to problems encountered during shipment and storage. There is a need of another study that would provide a full picture of species common in the study area.

In the current study brucellosis seropositivity was found to be higher in pastoral areas than in agropastoral areas but the difference was not statistically significant. In a study conducted in Chad by McDermont and Arimi (2002), brucellosis seropositivity was found to be higher in pastoralist communities than in other communities. However, the methodology used in the current study was not strictly random. In some households, the heads of households decided on who should be screened and in other households those who considered themselves to be

unwell presented to be screened for brucellosis. Children were scared of being bled, some ran away and some did not cooperate during the exercise making it difficult to screen them for brucellosis. This could have contributed to the difference in the findings of the studies.

4.6 Conclusion

There could be a considerable number of brucellosis cases in Arusha and Manyara regions that do not present to hospital due to various reasons. Using data from hospitals alone could therefore give an underestimation of the burden caused by brucellosis. Estimation of the burden caused by diseases such as brucellosis should also take into account members of the community that meet the diagnosis criteria. Community members should be made aware of the danger posed by goats in transmitting brucellosis particularly during handling fluids from goats such as blood and during assisting parturition. Future research should investigate the species of *Brucella* prevalent in Arusha and Manyara regions. Care should be taken in choosing the type of serological test to be performed in prevalence studies as some tests have shown to give false brucellosis prevalence.

5. Health-seeking behaviour and clinical characteristics of human brucellosis cases in Arusha and Manyara regions, Tanzania

5.1 Summary

Objectives: The aim of the study was to establish the clinical characteristics of human brucellosis cases in Arusha and Manyara regions and determine whether clinical criteria for diagnosis can be identified using the clinical features. The study also aimed to establish the health-seeking behaviour of brucellosis cases, to investigate the frequency of other conditions that may be confused with brucellosis, to determine treatment outcomes of brucellosis cases and to collect relevant data for assessing the burden caused by brucellosis.

Methodology: Socio-demographic, clinical and laboratory data were collected from patients who reported to selected hospitals between June 2002 and April 2003. All patients with conditions suspicious of brucellosis on the basis of preliminary clinical examination and history were enrolled into the study as brucellosis suspects. Blood samples were taken and tested at the hospitals using the hospital serological test, the supplied RBPT, and later the RBPT at the SUA and the c-ELISA test at the VLA. A follow-up of 49 cases was made to assess treatment outcome and nine cases for duration of *Brucella* antibody response.

Results: On the basis of hospital serological tests, brucellosis was diagnosed in 14.4% (n=1153) cases. Of the remainder, 26.4% were diagnosed and treated for malaria, 17.3 % for typhoid, 16.7 % for joint complaints, 6.3 % for other bacterial infections, 1.3% amoeba, 0.3 % tuberculosis and the remaining 17.3% were treated for other diseases such as worm infestation, diabetes, psychiatric disorders, low and high blood pressure, and other gastrointestinal complaints such as ulcers. Of the suspects 6.2% (n=1586), tested positive for the c-ELISA test, and were defined as brucellosis cases. The incidence of brucellosis was 11.2 cases /100,000 people per annum. Joint pain, headache, backache, fever and fatigue were the main clinical features described by the

confirmed c-ELISA positive patients, but these were also most commonly reported by the c-ELISA negative patients initially suspected as having brucellosis. It was therefore difficult to use clinical features for the definitive diagnosis of brucellosis. Different practitioners were found to prescribe different regimes for the treatment of brucellosis some of which are not recommended for the treatment of brucellosis and treatment outcome was poor. Patients with brucellosis delayed going to hospital with a median delay time of 90 days, and with 20% of the cases presenting to hospitals more than a year after the onset of symptoms. Distance to the hospital, keeping animals and knowledge of brucellosis were significantly associated with patient delay to present to hospital. Antibodies to *Brucella* were found to persist for a period of over 12 months after treatment for brucellosis. People engaging in business were much more common among the brucellosis cases than among suspects with other flu-like diseases.

Conclusion: More efforts need to be directed towards improving the diagnosis and treatment of brucellosis to reduce prolonged human suffering from brucellosis in Arusha and Manyara regions. This should include the evaluation and adoption of standardized diagnostic and treatment protocols. Health education should also stress the importance of early presentation to hospitals for prompt treatment. Practitioners should be made aware of the implications of persistent antibodies on the diagnosis and treatment of brucellosis in an area where other febrile conditions with similar clinical features co-exist. Future research work on brucellosis should try to work on thorough clinical examination and severity of clinical features brucellosis cases present with.

5.2　Introduction

Brucellosis is caused by gram-negative bacilli, of the genus *Brucella* (*Brucella abortus, B. suis, B. melitensis and B. canis*) (Young, 2000a). Clinical features include fever, fatigue, headache, sweating, joint pain, loss of appetite, muscular pain, lumber pain, weight loss, hepatomegaly, splenomegaly and arthritis. The multiple and non-specific features of brucellosis contribute to difficulties in the diagnosis of brucellosis in areas where diseases with similar clinical features such as malaria, tuberculosis, typhoid and joint diseases co-exist (Colmenero, *et al.*, 1996; Young, 2000a).

In many sub-Sahara African countries, febrile or flu-like conditions with similar manifestations occur commonly and have significantly contributed to difficulties in the diagnoses of such diseases as brucellosis, typhoid, malaria, amoeba and tuberculosis (Mutanda, 1998; Maichomo *et al.,* 1998). In Narok, Kenya, 12% of flu-like patients were diagnosed using the RBPT as brucellosis patients and 40% typhoid (Maichomo *et al.*, 2000). In Kampala, Uganda, of patients with joint pain, general malaise, and/or constant headache, 73% were found to be suffering from malaria and 13.3% from brucellosis (Mutanda, 1998).

As it is difficult to diagnose brucellosis clinically, its diagnosis relies on laboratory investigations (Schwabe, 1984; Maichomo *et al.,* 2000). However, most of the hospital laboratories in rural sub-Saharan Africa have limited capacity for the diagnosis of brucellosis. Brucellosis is commonly diagnosed after failure to respond to malaria, typhoid or tuberculosis treatments (Oomen and Waghela, 1974; Muriuki

et al., 1997). In Tanzania, it was observed that serological diagnosis was only conducted in districts or designated district hospitals (Chapter 2). In Kenya, local clinics were conducting the RBPT, but additional tests such as the SAT were only conducted in central veterinary or medical testing facilities (Muriuki *et al.,* 1997; Maichomo *et al.,* 2000). Most of these facilities however are not easily accessible to the majority of people in rural areas of Africa due to their geographical location and poor infrastructure.

Although generally speaking, any member of the public is at risk of getting brucellosis through consumption of poorly prepared dairy products in the form of meat, milk, cheese and butter, certain occupations such as veterinarians, butchers, abattoir workers, meat inspectors, farmers and those working in meat packing and dairy processing industries are known to be at a greater risk (McDermott and Arimi, 2002; Smits and Cutler, 2004). In South Africa for example, brucellosis caused five out of seven occupational infections of abattoir workers (Mauff, 1980). In Ethiopia, brucellosis contributed to the same proportion of occupational infection amongst veterinarians and farm workers (Seboxa, 1982). Although the number of cases that were examined was few, the results shed some light on brucellosis as an occupational hazard and challenge further research on brucellosis as an occupational hazard.

Brucellosis caused by *B. melitensis* is the most important clinically apparent disease in humans and is the one usually associated with occupational exposure or consumption of poorly prepared dairy products (Corbel, 1997), followed by infection

with *B. abortus* and by *B. suis* (Domenech *et al.*, 1983). Because of its greater severity, infections with *B. melitensis* are generally considered more likely to be diagnosed than infection with other *Brucella* species.

Health-seeking behaviour of patients suffering from infectious and non-infectious diseases in many parts of Africa have been analysed. These include those suffering from tuberculosis (Wandwalo *et al.*, 2000; Odusanya and Babafemi, 2004; Yimer *et al.*, 2005; Kiwuwa *et al.*, 2005), sleeping sickness (Odiit *et al.*, 2004), rabies (Kaare, 2006) and non-infectious diseases (Mwende *et al.*, 2005). Factors that determine when patients get hospital treatment vary from the patients' own reasons to those due to health providers. Patient factors include expectations that the symptoms might improve, visit to local traditional healer and self medication from a nearby drug shop or private clinic. Other factors include distance to the nearest health facility and socio-economic status. Some households are far from hospitals and poor infrastructure make accessibility to health care difficult. In areas where there is transportation, affordability of the costs of transport made patients unable to present to hospitals in time. Factors related to health provider included poor referral system, high work load and diagnostic difficulties (Wandwalo *et al.*, 2000; Odusanya and Babafemi, 2004; Yimer *et al.*, 2005; Kiwuwa *et al.*, 2005; Mwende *et al.*, 2005). The present study aims to investigate the duration and nature of clinical features of human brucellosis, to determine whether they can be used to establish diagnostic criteria for brucellosis and to examine the health-seeking behaviour, treatment outcome, and frequency of other conditions that may be confused with brucellosis. Data for estimations of burden caused by brucellosis were also collected during this study (see chapter 8).

5.3 Materials and methods

5.3.1 Study area, suspects and cases enrolment

The study was conducted in the northern regions of Arusha and Manyara, Tanzania, comprising the districts of Mbulu, Ngorongoro, Babati, Karatu and Hanang. Details of hospitals involved, their location and the methodology for data collection have been discussed in chapter 2. Briefly, the study group was drawn from patients presenting to the hospitals, between June 2002 and April 2003.

Plate 5. 1: A health centre in Simanjiro with patients waiting for investigation and treatment

All patients who presented to the hospitals with any of the clinical features including fever, headache, joint pain, malaise, backache, fatigue and loss of appetite were enrolled into the study as suspected brucellosis cases or suspects. Detailed data on clinical history, clinical features, impact of disease, location of household and potential risk factors (such as

occupation, age, sex) were collected by the practitioners and blood sampling for the RBPT (see section 2.4.5.1) and hospital tests were conducted. All patients with positive serological results for brucellosis conducted at the hospitals were named as 'brucellosis seropositives' to distinguish them from 'suspected brucellosis patients' and all patients with any two or more of fever, headache, joint pain, waist pain, backache, malaise, fatigue and tested positive with the c-ELISA test at the VLA (see section 2.4.5.2) were defined as brucellosis cases. Nine cases that received treatment for brucellosis were followed-up to investigate the persistence of antibodies to *Brucella*. The objective of the exercise was to determine how long cases will continue to test positive for brucellosis.

5.3.2 Incidence of brucellosis

The incidence of brucellosis in the study area was calculated from the total number of new confirmed cases of brucellosis recorded during the study period divided by the total population at risk in the study area. The study area was assumed to have a population of 1,054,238 people during the study period (Population and housing census, 2002). To get an annual estimate of incidence of brucellosis a cross-multiplication was done and later a factor of 100,000 was used to ease interpretation of incidence obtained.

5.3.3 Distance to the nearest hospital

The GIS coordinates of the households and of the hospitals were recorded from a hand-held Garmin® GPS machine. All coordinates were then transferred to Excel 2003 spreadsheets (Microsoft Corporation, Redmond, Washington, USA) and the distance between the

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hospitals and the households calculated in kilometers using ArcGIS 9 software (ESRI, Redlands, California).

5.3.4 Patient delay

Patient delay was the time interval between the development of first symptoms of brucellosis to the time presented to hospital. Thirty days since the onset of first symptoms was taken as the cut-off point during which any patient with brucellosis symptoms was supposed to have presented to hospital for diagnosis and treatment (Colmenero *et al.*, 1996). The outcome variable was therefore binary with all cases who presented to hospital 30 days or more after the onset of the first symptoms defined as delayed going to hospital and those who presented earlier than 30 days not delayed.

5.3.5 Data analysis

Data were entered on Excel 2003 spreadsheets (Microsoft Corporation, Redmond, Washington, USA) and analyzed using Minitab version 1.4 (Minitab Inc. 2000, Release 14 for Windows, State College, Pennsylvania). Chi-square tests were used to analyze all data and Fisher's exact test was used where 2x2 tabular results were obtained with any expected counts of less than 5. A p-value of <0.05 was considered statistically significant. In multivariate analysis, Minitab version 1.4 was used to run logistic regression analysis. A backward stepwise method was used to find the best suite of variables (model simplification). The least significant variables were considered first for removal. Any variables that caused an insignificant increase in deviance on removal

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from the model was left out of the model and the variable that caused a significant

increase in deviance on removal was retained in the model.

5.4   Results

5.4.1   Brucellosis suspects

Of the 2230 patients who presented at the hospitals with clinical features consistent with

brucellosis (brucellosis suspects), detailed clinical data were available from 1153 patients.

5.4.2   Brucellosis cases

Of the 1586 samples that were collected from suspected brucellosis patients over a

ten-month period and sent to Weybridge for the c-ELISA test, 98 (6.2%) tested

positive for brucellosis. These 98 cases were considered to be confirmed positive

cases, resulting in an annual incidence of brucellosis of 11.2 cases /100,000. The sex

and age distribution of cases is shown in Table 5.1.

Table 5. 1: Sex and age distribution of confirmed brucellosis cases

Age groups	Males	Females	Total	Percentage (%)
1-15	12	2	14	14.3
16-30	11	15	26	31.6
31-45	12	19	31	26.6
46-60	9	7	16	16.3
>60	9	2	11	11.2
Total	**53**	**45**	**98**	**100.0**

Among all patients who presented to the study hospitals, the highest proportion of brucellosis cases was found in Hanang district 13.6% (46/336) followed by Ngorongoro district 11.3% (18/159). The remaining districts recorded 6.7% (7/103) cases Mbulu, Karatu 5.5% (6/109) cases, and Babati 2.3% (21/888) cases (Table 5.2).

Table 5. 2: Distribution of brucellosis cases in districts and farming systems

District	Predominant Farming system	Number of cases	Number of all suspects	Percentage (%)
Babati	Agropastoral	21	885	2.4
Karatu	Agropastoral	6	106	5.7
Hanang	Agropastoral	46	338	13.6
Mbulu	Agropastoral	7	102	6.9
Total Agropastoral		**80**	**1431**	**5.6**
Ngorongoro	Pastoral	18	163	11.3
Total pastoral		**18**	**163**	**11.0**

5.4.3 Test for the association between brucellosis and age, sex, farming system and different occupational groups

5.4.3.1 Univariate analysis

Using the VLA c-ELISA results as a binary response variable, logistic regression analysis was done to analyse the relationship between brucellosis and age, sex, farming system and different occupational groups. All variables with p-value less than 0.05 were include in the multivariate analysis (Table 5.3).

Table 5. 3: Univariate analysis of association between brucellosis and age, sex, farming system and different occupation categories

Variable	Coefficients	P-value	OR	95% confidence interval	
				Lower	Upper
Age	0.00	0.88	1	0.98	1.02
Sex (Male vs female)	0.53	0.06	1.71	0.96	3.04
Pastoral/agropastoral farming system	0.77	0.04	2.17	1.02	4.64†
Employed	-0.31	0.59	0.73	0.22	2.39
Farming	-0.06	0.82	0.94	0.53	1.67
Housewife	0.16	0.73	1.18	0.45	3.05
Business	1.49	< 0.01	4.48	1.45	13.87†
Student	0.73	0.11	2.08	0.85	5.09
Teacher	-1.31	0.19	0.27	0.04	1.98

† - Variables included in the multivariate analysis.

5.4.3.2 Multivariate analysis of association between brucellosis, farming system and people engaging in business

In the multivariate analysis brucellosis was found to be associated with pastoral farming system and with people engaging in business (F=9.24, df=1, p<0.01) (Table 5.4).

Table 5. 4: Multivariate analysis of association between brucellosis, farming system and people engaging in business

Variable	Coefficients	P-value	OR	95% confidence interval	
				Lower	Upper
Pastoral/agropastoral farming system	1.62	0.02	5.07	1.62	15.83
Business	0.85	<0.01	2.36	1.10	5.08

5.4.4 Patient delay and treatment delay

Data on health-seeking behaviour were available for 49 brucellosis cases. Of these, 11 (23%) went to hospital within one month after the onset of symptoms, 10 (20%) between one and three months, 12 (25%) between three and six months, six (12%) between six months and one year and 10 (20%) sought treatment more than a year after the onset of symptoms (Figure 5.1). Using the cut-off point of 30 days as the time that a case was supposed to go to hospital, the median patient delay time was 90 days (mean, 157. 3 days). Health system delay was a result of false negative results causing a failure to diagnose 22 (54%) cases of brucellosis on their first visits to hospitals.

Figure 5. 1: The time cases presented to hospitals
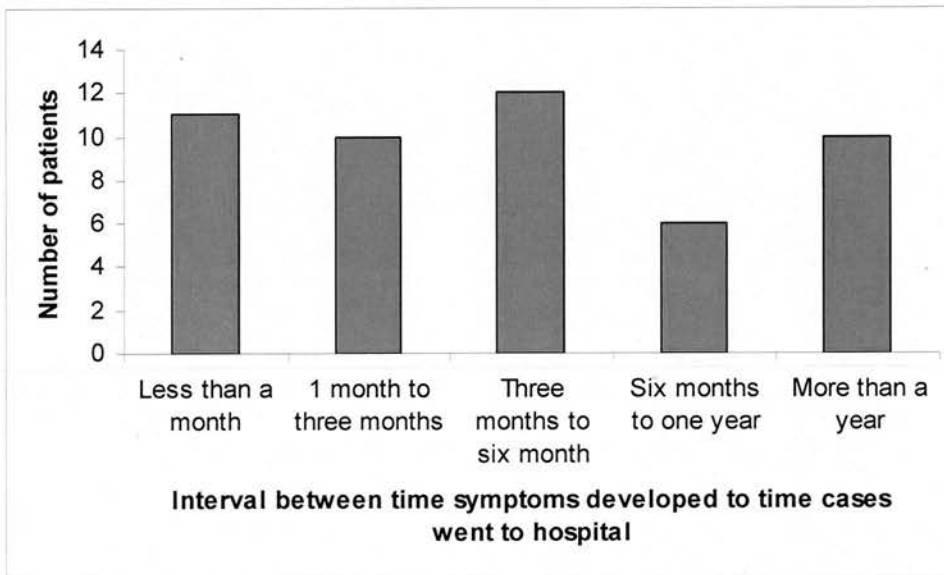
5.4.5 Factors responsible for patient delays

Using ArcGIS, the mean distance between the households of cases and hospitals was determined as 8.3 km, (median, 7.1 km). Univariate analysis showed that age of the case, distance to hospital, economic status, whether the household keeps livestock and knowledge of brucellosis were significantly associated with patient delay in presentation to hospitals (Table 5.5). Patient delay was found to be most associated with distance to the nearest hospital, knowledge of brucellosis and if the household of a case keeps livestock (likelihood ratio p-value 0.03) (Table 5.6).

Table 5. 5: Univariate analysis of factors responsible for patient delay

Variable	Coefficients	Std. Error	Odds ratio	95% confidence interval		Likelihood ratio p-value
				Lower	Upper	
Keeps livestock	1.61	0.63	5	1.45	17.27	0.003†
Distance to village centre	0.13	0.11	1.14	0.92	1.41	0.20
Religion	0.41	0.91	1.50	0.25	8.98	0.65
Tribe	0.69	0.71	2	0.50	7.99	
	1.25	0.80	3.50	0.73	16.85	0.14
Distance to hospital	0.11	0.06	3.12	1.00	4.26	0.02†
Gender	0.69	0.43	2	0.86	4.67	0.09
Age	0.02	0.01	1.02	1.01	1.04	0.001†
Knowledge of brucellosis	1.50	0.78	4.50	0.97	20.83	0.03†
Level of education	1.25	0.80	3.50	0.73	16.85	0.09
Economic status	1.47	0.64	4.33	1.23	15.21	0.02†
	0.69	0.87	2	0.37	10.92	
If any suffered from brucellosis	1.09	0.82	3	0.61	14.86	0.15

† - Variables included in multivariate analysis

Table 5. 6: Final model of multivariate analysis of causes of patient delay

Variable	Coefficients	Std. error	P-value	Odds ratio	95% confidence interval	
					Lower	Upper
Distance to hospital	0.03	0.07	0.00	2.23	0.89	3.18
If keeps livestock	1.23	1.11	0.04	3.42	0.39	30.02
Knowledge of brucellosis	0.28	1.09	0.02	1.32	0.15	11.34

5.4.6 Site of first treatment

The majority of brucellosis cases 43 (87.7%) gave a history of going to hospital as the first point of care, five (10.2%) purchased drugs from a nearby drug shop before going to hospital and one patient (2%) went to a local traditional healer first.

5.4.7 Clinical features experienced by suspects and cases

For comparative purposes, confirmed brucellosis cases were analyzed separately from all suspects to identify different characteristics of the cases that may help distinguish them from other febrile or flu-like conditions. Of the suspects (excluding brucellosis cases), 88.9% (n=1059) had joint pain, 82.1% backache and 65.8% headache. Other clinical features included fever 59.1%, fatigue 57.0%, chest pain 36.2%, abdominal pain 35.9% and loss of appetite 24.3%.

Of the cases, 43 (88.9 %) reported a history of joint pain, 41 (91.1%) backache, 38 (77.6%) headache, 36 (73.5%) fever, 26 (53.1%) fatigue, 17 (34.7%) loss of appetite, 15 (30.6%)

chest pain and abdominal pain was recorded from 14 (28.6%) cases. The summary of the

results above and p-values for the number of suspects and cases with a particular symptom

are included in Table 5.7.

Table 5. 7: Clinical feature experienced by confirmed brucellosis cases and brucellosis suspects, who were subsequently diagnosed as being seronegative and hence did not have brucellosis.

Symptom	No. of suspects	Percentage with symptom	Number of cases	Percentage With symptoms	χ^2 test p-value
Joint pain	816	88.3	43	88.9	0.91
Back pain	754	82.1	41	91.1	0.09
Headache	696	65.8	38	77.6	0.41
Fever	626	59.1	36	73.5	0.11
Fatigue	604	57.0	26	53.1	0.86
Chest pain	383	36.2	15	30.6	0.19
Abdominal pain	380	35.9	14	28.6	0.22
Loss of appetite	257	24.3	17	34.7	0.49
Respiratory symptoms	242	22.9	9	18.4	0.95
Neurological symptoms	116	10.9	9	18.4	0.30
Diarrhoea	107	10.1	3	6.1	0.60†
Lymphnode Enlargement	50	4.7	5	10.1	0.18†
Skin diseases	47	4.4	3	6.1	0.75†
Palpitations	2	0.2	0	0.0	0.48†

† - Fisher's exact test p-value

5.4.8 Differential diagnoses

Information on differential diagnoses was important in assessing the judgment of the practitioners as regards to diseases included for investigation i.e. to see which disease conditions were considered for diagnosis and which ones were not. Data on differential diagnoses was obtained from 1004 brucellosis suspects. These were divided into first, second and third differential diagnoses depending on what the practitioners thought were the most probable diagnoses in descending order (Appendix II). In the first category, malaria 435 (43.3%) was recorded by most practitioners as the first (most likely) diagnosis, followed by brucellosis 321(31.9%), typhoid 114(11.3%), arthritis 101(10.1%), urinary tract infection 9(8.9%), bronchitis 4(3.9%) (Table 5.8). Of the 321 suspected brucellosis patients whose first differential diagnosis was brucellosis, only 16 (3.98%) were confirmed as brucellosis cases.

Table 5. 8: Differential diagnoses of patients with clinical features consistent with brucellosis (brucellosis suspects).

First differential Diagnosis	Percent	Second differential Diagnosis	Percent	Third differential Diagnosis	Percent
Malaria	43.3%	Typhoid	35.0%	Brucellosis	50%
Brucellosis	31.9%	Brucellosis	33.6%	Arthritis	14.2%
Typhoid	11.3%	Malaria	16.7%	Malaria	10.7%
Arthritis	10.1%	Arthritis	6.3%	Typhoid	10.7%
Urinary tract infection	8.9%	Urinary tract infection	1.4%	Urinary tract infection	2.3%
Bronchitis	3.9%	TB	0.1%	Amoeba	1.9%

5.4.9 Hospital diagnoses of the cases presenting to the hospital with clinical features consistent with brucellosis (brucellosis suspects)

Of the 1153 suspects who were seen by practitioners, 304 (26.4%) were diagnosed and treated for malaria, 199 (17.3%) for typhoid, 192 (16.6%) for joint diseases which included back pain and waist pain and 166 (14.4%) were treated for brucellosis (referred to here as hospital brucellosis seropositives).

Suspects with other bacterial infections such as those involving the urinary tract, respiratory tract (excluding tuberculosis), gastrointestinal tract were 73(6.3%), amoebiasis 15(1.3%) and all forms of tuberculosis that was diagnosed in 4(0.34%) suspects. The remaining 200 (17.3%) were treated for other disease conditions such as worm infestation, diabetes, psychiatric disorders, low and high blood pressure other gastrointestinal complaints such as ulcers (Figure 5.2).

Figure 5. 2: Hospital diagnoses of the cases presenting to hospital with clinical features consistent with brucellosis (brucellosis suspects)
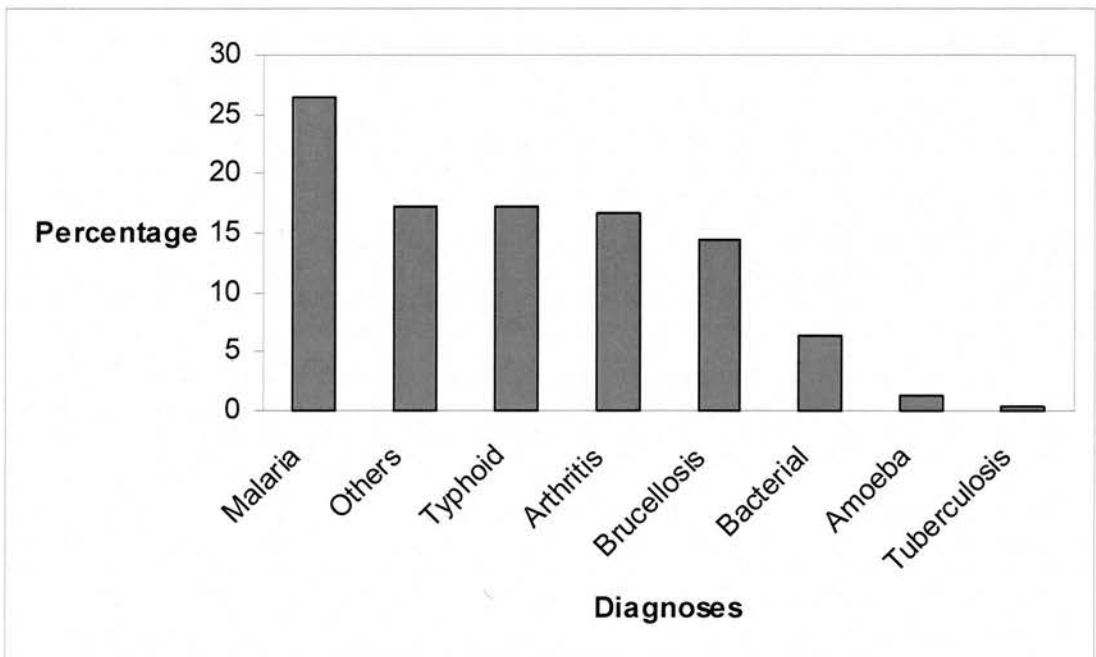
5.4.10 Mis-diagnosis

Of the 281 suspects who were diagnosed at the hospitals using the supplied Rose Bengal antigen and antigens used by the hospitals (referred to here as hospital brucellosis seropositives) and then treated as suffering from brucellosis, 238 (84.7%) tested negative with the c-ELISA test indicating that they were actually not suffering from brucellosis as a result of false positive diagnosis. On the other hand, of 997 suspects who were not diagnosed as suffering from brucellosis at the hospitals and were treated for other disease conditions, 40 (4%) tested positive with the c-ELISA test indicating that they were actually suffering from brucellosis (false negatives).

5.4.11 Progression of clinical features and treatment outcome

A follow-up of 41 cases was made to assess their treatment outcomes and the progression of their clinical features. The results showed that 19 (46%) patients were diagnosed and treated for brucellosis at the hospitals, while 22 (54%) were not diagnosed as suffering from brucellosis at the hospitals due to false negative results. False negative brucellosis patients comprised 17.1% patients who were treated for malaria, 15.2% for joint pain, 9.5% for typhoid, 4.9% for other bacterial diseases and 7.3% for non bacterial diseases such as gastric ulceration and worm infestation. All the cases that were treated for diseases other than brucellosis continued to have clinical features such as headache, joint pain, backache and general body malaise which are consistent with brucellosis.

Of the confirmed cases that were diagnosed and treated for brucellosis, 15.8% (3/19) did not show any signs of improvement after completion of brucellosis treatment, 10 (52.6%)

showed some improvements while the remaining 6 (31.6%) made a complete recovery

from brucellosis (Figure 5.3). Two cases (66.7%) that did not improve received

doxycycline tablets alone and one (33.3%) received doxycycline and cotrimoxazole. Four

cases (40%) that showed some improvement received doxycycline alone, three (30%)

received doxycycline and cotrimoxazole, one (10%) received floxacillin, one (10%)

received streptomycin and doxycycline and one (10%) received streptomycin and

cotrimoxazole (Table 5.9).

Figure 5. 3: Treatment outcome of brucellosis cases followed-up
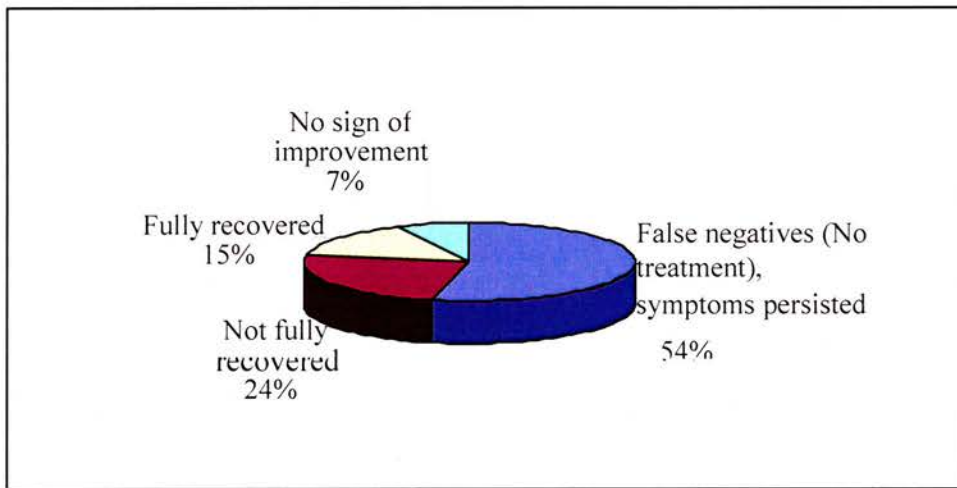
Table 5. 9: Brucellosis treatment regimes and outcome of treatment

Regime	Number of cases	Percentage
Cases that did not show any sign of improvement		
Doxycycline	2	66.7
Doxycycline and cotrimoxazole	1	33.3
Cases that showed some signs of improvement		
Doxycycline	4	40.0
Doxycycline and cotrimoxazole	3	30.0
Floxacillin	1	10.0
Streptomycin and doxycycline	1	10.0
Streptomycin and cotrimoxazole	1	10.0
Cases that completely recovered from brucellosis		
Doxycycline	2	33.3
Doxycycline and cotrimoxazole	2	33.3
Streptomycin and cotrimoxazole	1	16.7
Streptomycin and doxycycline	1	16.7

5.4.12 Duration of brucellosis clinical features and number of days cases spent in hospital as in-patients

The duration of brucellosis clinical features and the number of days that patients stayed in hospital as inpatients is discussed in chapter eight of this thesis.

5.4.13 Determination of persistence of antibodies to *Brucella* using the RBPT

A follow-up of nine brucellosis cases that received treatment for brucellosis was conducted to investigate the duration of antibodies to *Brucella* after treatment in relation to clinical features. Any with remission of clinical features, established by interview and physical examination, was considered to have recovered from brucellosis. Results showed that 6 (66.7%) cases continued to test positive on the last day of follow-up (Table 5.10). Only one patient (11.1%) completely recovered from brucellosis, the remaining 8 (88.9%), showed some improvement but still had some of the original clinical features.

Table 5. 10: Results of follow-up of clinical features and serostatus using the RBPT.

Id. No.	Village	Date of 1st test results and clinical features	Date of 2nd results and clinical features	Date of 3rd results and clinical features	Treatment received	Duration of antibodies
581	Oldonyo Wasso	14/3/03 +ve Cough, anorexia Joint pain, fever	17/08/03 +ve Symptoms free	10/1/04 +ve General weakness	Doxycycline (duration unknown)	>10 months
584	Oldonyo Wasso	14/3/03 +ve Joint pain, fever, Backache	17/08/03 +ve Headache, joint pain, malaise	10/1/04 +ve Intermittent fever	Doxycycline for 4 weeks and cotrimoxazole for 6 weeks	>10 months
652	Masakta	15/10/02 +ve General malaise, backache, Joint pain	10/09/03 +ve Flu, low grade fevers	20/2/04 +ve General body malaise, weakness	Doxycycline 6 weeks	>16 months
K4	Mang'ola	15/8/02 +ve Loss of appetite, fever, joint pain	18/09/03 -ve Abdominal distension	_____	Doxycycline 6 weeks	<13 months
775	Basutu	11/2/03 +ve Headache, Backache, fever joint pain	7/09/03 +ve Joint pain, fever, headache	19/1/04 +ve Joint pain	Streptomycin 4 weeks and Doxycycline 4 weeks	>11 months
809	Jorodom	15/6/02 +ve Headache, joint pain, backache, fever	9/09/03 -ve Symptoms free	22/2/2004 -ve Joint pain, weakness	Doxycycline for 6 weeks and Local herbs	<15 months
863	Oloipir	12/01/03 +ve General malaise and pain, wasted	15/8/03 +ve General malaise with some improvement	10/02/04 +ve Much better, weakness, joint pain	Doxycycline for 4 weeks and cotrimoxazole for 6 weeks	>13 months
800	Gitting	5/07/02 +ve Fever, backache, malaise, fatigue, joint pain	7/09/03 +ve General body malaise and backache	19/2/04 -ve Symptoms free	Cotrimoxazole and Streptomycin for 4 weeks	<14 months
950	Endasaki	14/4/03 +ve Fever, joint pain, vomiting, malaise, fatigue	9/09/03 +ve Symptoms free	18/2/04 +ve Had a bout of fever and headache	Streptomycin 4 weeks and Cotrimoxazole for 6 weeks	>10 months

5.5   Discussion

There were proportionally more cases of brucellosis recorded in hospitals located in pastoral than in hospitals located in the agropastoral areas. During cross-sectional survey, it was established that seropositivity was higher in the pastoral than in the agropastoral system although the difference was not statistically significant. Such factors as movement with livestock, animal husbandry and consumption habits of animal products among the people living in the pastoral system are likely to contribute to more cases in the pastoral systems (Schelling *et al.*, 2003). As the occurrence of the disease in humans is largely dependent on the animal reservoir (McDermot and Arimi, 2002) control strategies for brucellosis should be given priority to pastoral areas.

Only 23% of the cases started to seek treatment within a month since the onset of symptoms. More striking however, was the fact that 20% of the cases stayed for a period of over a year without seeking any treatment. Distance to the nearest hospital, keeping livestock and knowledge of brucellosis were significantly associated with delay to present to hospital. Cases that stay far from the hospitals were found to have a higher chance of delaying going to hospital than those staying close to hospitals. In the study area many households stay far from health facilities and the infrastructures are still poor. Delay to present to hospital is therefore associated with how accessible the hospitals are. Cases keeping livestock were also found to delay going to hospital. Probably it was not possible to leave their livestock with nobody to attend and go to hospital. They only presented to hospital when symptoms worsened. Cases with the knowledge of how brucellosis is transmitted and manifests also delayed going to hospital. It is possible they think brucellosis has mild acute clinical features and fatality is rare and only when the symptoms get worse do they seek medication.

The majority of cases first sought treatment at a hospital and a few either treated themselves at home by buying medicine from a nearby drug shop (10.2%) or attended a local traditional healer (2%). In the study area, a purely private health system has not been fully established, so the majority of patients go to public hospitals which are run by government or religious groups (missionary hospitals). It was therefore unlikely for cases to be delayed by commercially oriented health providers such as those found in urban areas. Studies conducted on the causes for tuberculosis patients delay to go to hospitals, established that patients spent longer periods seeking treatment from traditional healers or at commercial hospitals where most of their financial resources were used without getting proper treatment (Wandwalo *et al.*, 2000).

Brucellosis still poses a significant diagnostic challenge in the study area. The clinical features that brucellosis cases presented with were found to be similar to those recorded from suspects with other febrile conditions, with joint pain, backache, headache, fever, and fatigue being the most common features. None of the clinical features was found to be statistically more common in either of the groups than the other. Co-existence of such diseases in the study area therefore, makes it difficult to use clinical features to reach the definitive diagnosis of brucellosis. Practitioners in the study area should therefore consider testing routinely for brucellosis all patients presenting with non-specific clinical features such as joint pain, headache, backache, fever, fatigue and malaise. Similar conclusion was reached by Canova *et al.,* (1993) in a study conducted in Yugoslavia. Brucellosis patients were found to present with nonspecific manifestations which caused diagnostic delays. It was suggested that high index of suspicion is important in all patients presenting with multiple nonspecific features for early diagnosis of

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brucellosis in rural areas where dairy products are commonly used and brucellosis has been reported.

The finding that there were more people engaging in business among the cases than among the suspects suggests that practitioners should give brucellosis a high index of suspicion when a person doing business presents to hospital with non-specific flu-like clinical features. Data on the types of businesses cases were doing were not collected so it was difficult to establish how the type of business they were doing could have put them at risk. However, in the study area, people engaging in business meet in market places and in auctions where they consume among other things meat and milk. In auctions, animals are slaughtered and the meat cut into portions for selling or roasting. It possible that handling and consumption habits of animal products amongst the people engaging in business expose them to the risk of getting brucellosis. The majority of individuals in the study area are farmers. It is possible even teachers, students, those doing business are farmers of some kind as well, this explains why farming did not appear as a risk occupation in the analysis. Occupationally at risk people such as meat inspectors, butchers, abattoir workers and veterinarians who are known to be more at risk of brucellosis (Serra et al., 2000; Thakur and Thapliyal, 2002; Karimi et al., 2003; Reid, 2005) were not recorded in either of the groups. It is possible that they are very few in the study area and none of them fell sick during the study period or they are more aware of diseases such as brucellosis, so they protect themselves against them.

As a result of false negative results, 22 (54%) brucellosis cases were not diagnosed as suffering from brucellosis on their first visit to hospitals, they were treated for other diseases hence they

continued to suffer from brucellosis. The findings agree with Muriuki *et al.*, (1997) and Oomen and Waghela (1974), who found that brucellosis cases are normally diagnosed after failure to respond to treatment for malaria and typhoid. Apart from poor diagnostic tools, practitioners have always been underestimating the threat posed by brucellosis. Patients presenting with non-specific clinical features are not tested for brucellosis routinely. In the current study, 31.9% of the suspects were considered for brucellosis investigation as the first differential diagnosis. Out of these only 3.9% were confirmed as brucellosis cases. However, the results that brucellosis was second to malaria in terms of consideration for investigation could have been caused by the Hawthorne effect (Roethlisberger and Dickson, 1939, cited by Holden, 2001). It was evident in the preliminary hospital survey (see chapter 3) that brucellosis is not routinely tested for in patients presenting to hospitals with non-specific symptoms. The fact that the study was conducted by the brucellosis study team could have influenced the judgment of the practitioners.

Although brucellosis false seropositivity was higher (84.7%) than false negativity, it did not seem to pose a significant therapeutic problem for other flu-like conditions. Other flu-like conditions such as malaria and typhoid were found to be tested routinely on patients first presentations to hospitals. Since false positives received a combination of antibiotics and pain killers with an intention of treating brucellosis, they tended to get a relief of symptoms for the flu-like conditions they were suffering from. Hence, except for patients suffering from tuberculosis and amoeba, most of brucellosis false positives received treatment for their conditions without a definitive diagnosis.

Of the 19 cases that were diagnosed correctly and received treatment for brucellosis, only six (31.6%) had a complete recovery and three (15.8%) did not show any sign of improvement. The persistence of clinical features in cases could mean failure to respond to treatment, re-infection, relapse or co-existence of untreated other flu-like conditions. In a study conducted by Ariza et al., (1995) the choice of less effective antibiotics was found to be among the factors causing relapse of brucellosis. Since Brucella localize within the host's reticulo-endothelial cells, a site relatively inaccessible to antibiotics (Khan et al., 1989; Hall, 1990), proper combination of antibiotics for longer periods is required to improve the outcome and prevent relapses (Ariza et al., 1985). Practitioners in the hospitals involved with the study did not adhere to recommended brucellosis treatment regimes, this could have caused the high rate of treatment failure.

Based on the available data, it was difficult to ascertain the efficacy of different brucellosis treatment regimens. Practitioners did not use specific standardized treatment regimens for brucellosis, only available drugs were prescribed. As a result, for 19 cases that received treatment for brucellosis, five treatment regimens were used some of which are not recommended for brucellosis treatment. A better planned clinical trial needs to be carried out with more number of cases assigned specific treatment regimens and treatment groups followed-up at fixed time intervals for assessment.

Antibodies to Brucella were found to persists for more than 12 months after treatment. Similar findings were established in South Africa by Mauff (1980) who found that antibodies to Brucella could persist for a period of up to 12-24 months after cure and posed diagnostic difficulties for brucellosis and other flu-like conditions. It is likely that some cases of malaria, typhoid,

tuberculosis or other joint diseases are unnecessarily diagnosed and treated for brucellosis because of persistent antibodies to *Brucella*. Clinicians should be aware that seropositivity to *Brucella spp* using current hospital diagnostic tests is insufficient evidence to provide a confirmed diagnosis of brucellosis and that other differential diagnoses should be excluded.

In the present study thorough examination of the cases to rule out organ involvement such as the heart, liver, spleen and lungs and any neurological involvement which have been shown to be common in human brucellosis (Charters, 1980; Fallatah *et al.,* 2005; Troy *et al.,* 2005), were not conducted. In addition, the assessment of the severity of clinical features with which brucellosis cases presented to hospitals was not recorded. Some of brucellosis clinical features could have been missed by this omission. Future research work should take into account the severity and pathognomonic features of brucellosis so as to get a full picture of brucellosis cases in Tanzania. Further examination using extra facilities such as ultra sound, X-ray and extra blood tests such as full blood picture and organs function tests could be performed. Because most of these tests are not performed in the rural hospitals, arrangements could be made for them to be performed elsewhere for scientific and epidemiological knowledge.

5.6 Conclusion

More efforts need to be put on improving the diagnosis and treatment of brucellosis as failure to diagnose and treat brucellosis contributed to prolonged human suffering. This should include adoption of standardized diagnostic and treatment protocols for brucellosis. Evaluation of different tests with an intention of coming up with the tests with high sensitivities and specificity and a well planned clinical trial of different brucellosis drug therapies need to be conducted.

Among other important issues, health education to the public should also include emphasis on patients to go to hospital early when chronic clinical features and complications have not developed as late presentation may carry poor prognosis even after treatment. Practitioners should also be made aware of the implications of persistent antibodies to *Brucella* on diagnosis and treatment in an area where other febrile conditions of similar clinical features co-exist.

6. Risk factors for transmission of brucellosis to humans in Tanzania

6.1   Summary

Objective: To determine the risk factors for transmission of brucellosis to humans in Tanzania.

Study design: A matched case-control study.

Methodology: Any person with a positive result by the c-ELISA test for brucellosis, and presenting to the selected hospitals with at least two clinical features suggestive of brucellosis such as headache, fever which is recurrent or continuous, sweating, joint pain, joint swelling, general body malaise or backache, was defined as a case. For every case in a district, a corresponding control was traced and matched by sex using multistage sampling. Other criteria for inclusion as a control included that the matched individual had a negative c-ELISA test result and would present to hospital if sick. Using risk sets as a matching variable, and cases and controls as outcome variables, models were fitted to test their significance and the likelihood ratio was used to identify risk factors for human brucellosis.

Results: Multivariable analysis showed that brucellosis was associated with assisting cattle, sheep or goat during abortion. It was shown that the closer the distance between households, the higher the risk of brucellosis. People who were of Christian religion were found to have a higher risk of brucellosis compared to other religions. No association was established between human brucellosis and brucellosis serostatus of their own livestock. In humans, there was no association between human brucellosis and HIV serostatus.

Conclusion: Protecting humans against contact with fluids and tissues during assisted parturition of livestock may be an important means of reducing the risk of transferring brucellosis from livestock to humans. The possibility of contracting brucellosis from neighbors or other herds should also be considered. These can be achieved through

health education to the communities where brucellosis is common. There was no

association between HIV serostatus and brucellosis cases, indicating that brucellosis is

not more likely to occur in patients immune compromised by HIV in the study areas.

6.2   Introduction

Brucellosis is a zoonosis of veterinary, public health and economic significance in most developing countries (WHO, 1997). Human brucellosis is a severely debilitating disease that requires prolonged treatment with a combination of antibiotics leaving permanent and disabling sequelae, and results in considerable medical expenses in addition to loss of income due to loss of working hours (Corbel, 1997; Smits and Cutler, 2004; Solera *et al.,* 1997). In livestock, brucellosis results in reduced productivity, abortions and weak offspring and is a major impediment for trade and export. Almost all domestic species can be affected. Thus, its prevention, control and eradication are a major challenge for public health programmes (Corbel, 1997; Smits and Cutler, 2004). The disease has been eradicated in a number of countries, including the UK, since 1980-81. Even in these countries, human infections are still encountered as the occasional case arising from endemic areas (WHO, 1997).

Where brucellosis exists in sheep and goats, it causes the greatest incidence of infection in humans (WHO, 1997). Most human cases involving field strains of *Brucella* species can be traced to domestic food animals, and the prevalence of disease in humans reflects its occurrence in livestock reservoirs. Commonly, *B. abortus* and *B. suis* infections are associated with certain occupational groups, including farm workers, veterinarians, and meat-packing employees (Young, 1983). Transmission through consumption of contaminated dairy products is the route that has been well documented in many parts of the world (Busch and Parker, 1972). Unpasteurized milk and processed dairy foods from infected animals have been considered a source of infection

for the general population, and infected carcasses as a source of infection for workers in the meat-packing industry.

Veterinarians may acquire brucellosis from assisting births in infected livestock, as well as through inadvertent exposure to vaccines. Veterinarians, laboratory staff, and workers based in meat plants were found to be at increased risk of exposure in Ireland and Spain (Reid, 2005; Bouza *et al.*, 2005). In Burundi, the prevalence of positive serology was found to be significantly higher in professionally at risk people than in people consuming contaminated food (Laroche *et al.*, 1987). Airborne transmission of bacteria to humans has also been documented in clinical laboratories and abattoirs (Buchanan *et al.*, 1974). Human-to-human transmission through sexual intercourse has been reported by Ruben *et al.*, (1991).

Contact with products of conception has been shown to be an important factor in the transmission of brucellosis to humans (Young, 1983). In Kyrgyzstan, brucellosis was shown to be associated with exposure to aborted farm animals in the household and consumption of home-made milk products obtained from bazaars or neighbors (Kozukeev *et al.*, 2006). Touching calves or placentas that were infected with the *Brucella* species was found to be associated with brucellosis transmission during cattle birth in Korea (Lim *et al.*, 2005). Similar findings were obtained in Greece, Chad and Saudi Arabia (Cooper, 1992; Bikas *et al.*, 2003; Schelling *et al.*, 2003) where products of conception, especially the placenta were found to be a risk factor for brucellosis transmission.

The Tanzanian economy depends largely on agriculture, of which livestock forms an integral part (Ministry of Agriculture and Cooperatives, 1995a). As the economic future of the country lies mainly in agricultural development, diseases with considerable effect on livestock productivity and human health such as brucellosis should not be ignored (Stark and Protz, 1973). The objective of this study is to explore factors that are responsible for transmission of brucellosis to humans in Arusha and Manyara regions and identify the most plausible ways to minimize the transmission of the disease from animals and their products based on the established risk factors. This will hopefully decrease the burden caused by brucellosis.

6.3   Materials and methods

6.3.1   Study area

Details of the study area and the methodology used have been discussed in chapter 2 of this work. A brief account is given.

6.3.2   Study design

This was designed as a matched case-control study. All patients who presented to the selected hospitals between July 2002 and June 2003 with clinical features suggestive of brucellosis were recorded and investigated.

6.3.3   Selection of cases and controls and blood sampling

Any person with a positive serological result by the c-ELISA test conducted at the VLA, and showing at least two of the following clinical features: headache, fever which is recurrent or continuous, sweating, joint pain, joint swelling, general body malaise or backache, was defined as a case. For every case, a community-based control was selected. A control was an individual in the same district as the case, having a negative brucellosis serological result by the c-ELISA test, matched by sex and coming from a hospital-going household.

Blood sampling and testing for brucellosis in both humans and livestock was conducted using the RBPT (Davies, 1971) section 2.4.5.1 in the field and at the SUA, the c-ELISA test for brucellosis (Perret, *et al.*, 2001) section 2.4.5.2 at the VLA and testing using Vironostica Uniform II *Plus* O, at Muhimbili University College of Health Sciences in Dar es Salaam were conducted. A questionnaire on potential risk factors was also administered during the follow-up period (Appendix I).

6.3.4   Data analysis

A conditional logistic regression was performed to analyse case-control data using Egret for Windows version 2.0 (Cytel Software Corporation, Cambridge Massachusetts). The univariable relationships between all independent variables and brucellosis were estimated by including them individually in a model with brucellosis serological result as the dependent variable. Forty-four risk sets were obtained from the cases and controls studied. Using risk sets as a matching variable, and cases and controls as outcome variables, a number of models were fitted to test their significance as risk factors for a brucellosis positive serological result.

Due spillage and small amount of sample collected, only 66 samples from cases and controls were available for HIV testing. The univariate analysis using HIV results was therefore carried out separately using Epi-info 6 and the results were interpreted separately without being included in the multivariate analysis. All the 95% confidence intervals were calculated using Epi Info 6 software (CDC, Antanta, Georgia).

Multivariable models were created by a backward stepwise procedure using Egret for windows. Variables that had a p value of ≤ 0.2 from the univariable analysis were considered for inclusion in the final models. Values were retained if on their removal there was a significant increase of the residual deviance of the model with likelihood ratio statistics (LRS) of $p > 0.05$ and they were removed from the model if they caused an insignificant increase or decrease in the residual deviance with LRS of $p < 0.05$.

6.4   Results

6.4.1   Cases and controls

Of the 98 cases identified in hospitals, only 44 were available for follow-up (Table 6.1). Of the 44 cases, 25 were males and 19 were females. Four cases died before a follow-up was conducted; these included two patients from Hanang district, one from Karatu district and one from Ngorongoro district. A total of 55 controls were followed-up (Table 6.1) during the study period, of these 29 were males and 26 females. The mean age for the cases was 36.4 and for controls 36.3 with standard deviations of 17.8 and 17.3 respectively. The locations of the cases and controls followed are as shown in Figure 6.1.

Table 6. 1: Cases and controls followed-up

	Babati	Hanang	Karatu	Mbulu	Ngorongoro	Total
Case	11	24	1	1	6	44
Control	12	34	1	1	8	55
Total	**23**	**58**	**2**	**2**	**14**	**99**

Figure 6. 1: Map of north-eastern Tanzania showing locations of cases, controls and hospitals in Arusha and Manyara regions.
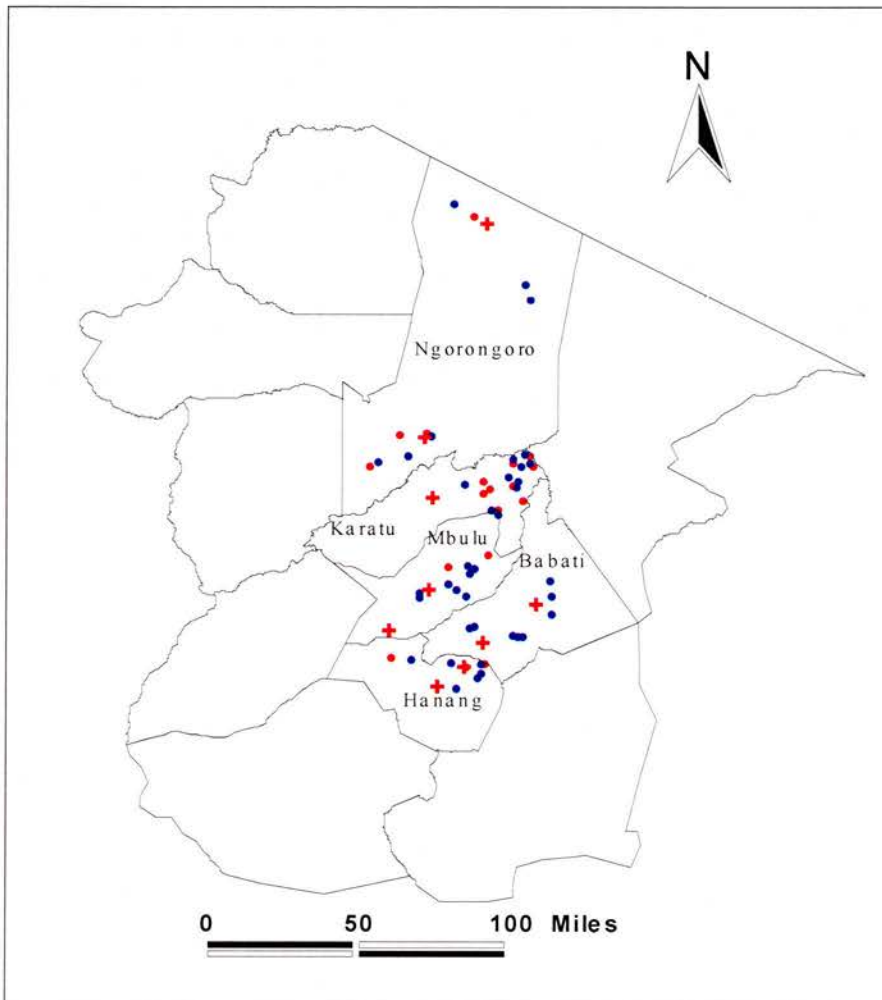
Key

+ - Hospital

● - Control

● - Case

6.4.2   Univariable analysis

Univariable relationships between independent variables and a brucellosis cases result are shown in Table 6.2. The likelihood of becoming a brucellosis case increased with any animal abortion, history of household member suffering from brucellosis and decreasing distance to the nearest neighbour irrespective of neighbour's serostatus.

The likelihood of brucellosis also increased with Christian religion, households where a goat had aborted, involvement in preparing meat, involvement in assisting aborting livestock and a middle socio-economic status.

Table 6. 2: Univariable relationships between independent variables and brucellosis cases

Variable	Coefficient	Standard error	p-value	Odds ratio	95% Confidence Upper	Lower	LRS p-value
Distance to the nearest neighbour's house -continuous	-0.01	0.004	0.02	0.99	0.98	0.99	0.007
Christian religion	2.81	0.78	0.07	4	1.03	13.46	0.04
Any member suffered from brucellosis	1.72	0.79	0.03	5.61	1.19	26.28	0.01
Abortion of any animal	1.27	0.46	0.006	3.55	1.43	8.80	0.003
Goat aborted	1.07	0.44	0.01	2.90	1.23	6.86	0.009
Involved in assisting abortion	1.49	0.65	0.02	4.46	1.25	15.92	0.009
Prepared any meat	0.96	0.48	0.04	2.62	1.02	6.74	0.03
Economic status-Low	0.80	0.71	0.26	2.24	0.55	9.05	
Economic status-Middle	2.51	0.95	0.008	12.31	1.92	78.79	
Economic status-High	0.16	1.06	0.88	1.17	0.15	9.39	0.008

6.4.3 Multivariable analysis

Three variables were included in the final model (Table 6.3). Brucellosis was significantly associated with involvement in assisting with abortion, with proximity to the nearest neighbor and with the Christian religion (likelihood ratio statistics p< 0.001).

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Table 6. 3: Multivariable relationship between independent variables and brucellosis

Likelihood ratio test	Coefficient	Std.Error	p-value	Odds Ratio	95% confidence interval	
					Lower	Upper
Distance of house to nearest neighbour's house Continuous	-0.02	0.01	0.01	0.98	0.97	0.99
Involved in assisting abortion	2.06	0.84	0.01	7.86	1.51	40.87
Christian religion	1.94	0.98	0.03	3.03	2.11	18.44

6.4.4 Result of HIV testing

Only 66 samples were available for HIV testing using Vironostica Uniform II *Plus* O (bioMérieux bv, Boxtel, The Netherlands), including 37 controls and 29 cases (Table 6.4). Of the samples tested, two cases tested positive for HIV and three controls (3.1%, 95% CI, 0.2-17.9) tested positive for HIV (OR, 0.84, 95% C I, 0.13-5.39).

Table 6. 4: HIV ELISA results of human cases and controls

	ELISA		
	Positive	**Negative**	**Total**
Case	2	27	29
Control	3	34	37
Total	**5**	**61**	**66**

Plate 6. 1: Assisting an aborting animal was found to be a risk factor for transmission of brucellosis to humans

Plate 6. 2: The closeness of the households in the study area is a risk factor for transmission of brucellosis to humans

6.5   Discussion

Brucellosis in humans was found to be associated with assisting aborting livestock. An abortion storm in a herd of livestock is among the common features of brucellosis in livestock (Schelling *et al.,* 2003). During abortion, large numbers of *Brucella*e are released which may, in turn, cause the infection to other animals in the herd (Manthei and Carter, 1950). The univariate analysis, showed that brucellosis was associated with a household where a goat had aborted, however it was not shown whether assisting goats during abortion was associated with brucellosis. The findings agree with Young (1983) who found that persons usually become infected with *Brucella* through direct contact with infected animals or their products. Place of residence, professional occupation with animals, and human's trauma during animal delivery were found to be important risk factors for brucellosis transmission to humans in Greece (Bikas *et al.,* 2003).

Contact with placenta of livestock was found to be highly associated with brucellosis transmission in a study conducted in Chad (Schelling *et al.,* 2003). In Saudi Arabia, assisting animals during parturition was found to be an important risk factor for brucellosis transmission, but no significant risk associated with other direct (unspecified) animal contact was observed (Cooper, 1992). Though there was no classification of outcome of parturition i.e. normal or abortion, or whether the placenta belonged to a live normal delivery or aborted material the studies point to the fact that products of conception are important risk factor for brucellosis transmission.

Distance between households was found to be significantly associated with brucellosis transmission. The shorter the distance between the households the greater was the chance of contracting brucellosis, irrespective of the serostatus of the neighbours. In most African communities, neighbors assist each other in conducting different activities. These range from home-based to farm-based duties. Assisting neighbour's animals during parturition, sharing of food stuffs such as milk and other dairy products is also not uncommon in communities visited in Arusha and Manyara regions. It is likely that some of the cases acquired brucellosis either while assisting neighbors' animals during abortion or during preparing meat.

The association between brucellosis and people belonging to Christian religion was rather unexpected in this study. There are many practices however that could be linked with the religious group in Arusha and Manyara regions that need be taken into consideration or need further investigation as far as the risks of contracting brucellosis are concerned. Issues like animal husbandry, the number of livestock kept, the location, interaction between livestock and humans and taboos associated with certain religious groups could be important factors for comparison in the study area. For example, in the study area, Muslims either do not keep animals or keep a few of them. It is also known that Muslims do not consume meat from an animal they do not know how it was slaughtered which means that Muslims would not consume meat in auctions or market places as much as the Christians.

Brucellosis in humans is a chronic disease with multiple presentations some of which are non-specific and could mask HIV in HIV endemic areas. In the current study there was no positive association between HIV and brucellosis in the study area (OR=0.84). This could be due to the low prevalence of HIV in the area or the number of samples tested was too few to allow conclusions to be drawn about the relationship between the serostatus of brucellosis in livestock and that in humans. The findings are similar to the study conducted in Kenya by Paul *et al.*, (1995) in which it was established that there was no association between *Brucella* antibody status and HIV status hence *Brucella* serology may be helpful in the diagnosis of patients with non-specific symptoms in East Africa, regardless of HIV status. A study carried out in Spain by Moreno *et al.*, in 1998 established that HIV infection does not seem to increase the incidence of brucellosis. Since most cases occur in asymptomatic patients with relatively normal immunity, the epidemiology, clinical presentation, diagnosis, response to therapy, and outcome are similar to those observed in non-HIV infected people.

In other parts of Africa brucellosis transmission was associated with a wide range of other risk factors, but all are animals to humans directly through contact or animal products to humans through consumption. In Nigeria the highest prevalence (20%) of brucellosis was observed among cattle handlers followed, in decreasing order of prevalence by goat rearers (10%), mixed sheep and cattle rearers (9%), mixed sheep and goat rearers (8%), and 4% among each of sheep rearers and non-rearers of animals (Baba *et al.*, 2001). The social habit of eating raw meat, e.g. raw liver or other offal with spices (Marrara or umfitfit) was found to be an important epidemiological factor in contracting

the disease in central Sudan, the majority of the patients were found to have a combined infection of both *Brucella abortus* and *Brucella mellitensis* (Mohd, 1989). The present study and others conducted in sub-Saharan Africa indicate that transmission of brucellosis to humans is primarily from livestock reservoirs. Thus, the risk to humans is a function of the risk in livestock and human-livestock effective contact rate (McDermott and Arimi, 2002; Baba *et al.*, 2001).

6.6   Conclusion

Health education on ways to prevent brucellosis transmission through contact while assisting animal delivery should be provided in animal keeping communities. Though it may be difficult for each and every farmer to obtain gloves to use while attending to livestock that are about to give birth, simple plastic bags that are easily and cheaply available in the rural settings could be used to prevent the direct contact between humans and the products of conception that have been shown to harbour *Brucella* species. During grazing far from home, pieces of clothes can be used to reduce contact with products of conception as well. Health education on possibilities of getting infection from neighbour's animals or consumption of dairy products from a neighbour should be taken into account as well. Dairy products such as milk and meat should be properly handled, thoroughly cooked or boiled to prevent the transmission of not only brucellosis but also other zoonoses such as tuberculosis.

As no association between brucellosis and HIV was established, the *Brucella* sero diagnosis could be an important diagnosis for febrile illness even in areas where HIV is

thought to be prevalent in Arusha and Manyara regions. Each disease condition should be treated on its merit. Patients presenting with clinical features suggestive of relapsing brucellosis should however be investigated for HIV.

7. **Evaluation of diagnostic tests for human brucellosis in Arusha and Manyara regions, Tanzania**

7.1 Summary

Objectives: To evaluate the diagnostic tests for brucellosis in Arusha and Manyara regions.

Methodology: Samples for evaluation of diagnostic tests were obtained from the cross-sectional study and hospital clinical study. In the cross-sectitonal survey, subjects for blood sampling were obtained by multi-stage cluster sampling to household level. At the randomly selected households, all family members who agreed to participate with the study were tested for brucellosis using the RBPT. In the clinical study, all brucellosis suspects were tested for brucellosis using antigen purchased by the hospital and Rose Bengal antigen supplied by the project. The RBPT was also performed at the SUA laboratory. Samples were shipped to the VLA for the c-ELISA test which was considered the gold standard test.

Results: The sensitivity and specificity of the RBPT in the cross-sectional survey were 39.4% and 98.8% respectively, at the laboratory, 38.7% and 96.8% respectively and at the hospitals, 44.3% and 89.5% respectively. All the other tests performed at the hospitals had sensitivities less than 76%, with Eurocell A[®] having the highest (75%). The specificities of the tests performed at the hospitals were also low with Eurocell A[®] having the highest (90%). There was a poor agreement between the RBPT performed at the SUA, the RBPT performed in cross-sectional survey and tests performed at the hospitals.

Discussion and conclusion: The antigens used for the diagnosis of brucellosis showed very low sensitivity. The results indicated that brucellosis is likely to be under-diagnosed in Arusha and Manyara regions. The storage of samples during transportation, errors

during testing and labelling and strain variation could also have contributed to the very low sensitivity results. The specificity of the RBPT was high but the specificities of antigens used at the hospitals were generally low. The diagnosis of brucellosis needs to be improved in Arusha and Manyara regions. There is a need to have a standardized protocol for diagnosis of brucellosis which should include the control of a number of antigens used and regular quality assurance surveillance. Zonal specialized laboratories might assist to improve brucellosis case detection.

7.2   Introduction

Many human diseases are under-diagnosed in Africa because of limited diagnostic capacity and coverage of the health services. The problem of under-diagnosis is more common among the poor people as a result of their uneven geographical distribution, and inherent difficulties in diagnosing diseases in rural and peri-urban areas (Schwabe, 1984). As a consequence, the under-diagnosis of such diseases as zoonotic diseases has greatly contributed to their under-reporting in developing countries, in particular, sub-Sahara African countries. A good example is shown by the official figures of incidences of rabies, brucellosis and trypanosomiasis in developing countries, they represent only a fraction of the actual disease burdens in these countries (Cleaveland, *et al.*, 2002; WHO 1998b; Cattand *et al.*, 2001).

Clinical features of brucellosis in humans are similar to malaria and typhoid (Mutanda, 1998; Muruiki *et al.*, 1997), it is therefore possible that some cases of brucellosis are recorded as malaria or typhoid where these diseases coexist. Diagnostic difficulties are also encountered with human African trypanosomiasis (HAT) and tuberculosis. HAT is difficult to diagnose because the parasites are not always evident in blood, and screening tests often generate a high proportion of false positives. In the case of tuberculosis, few hospitals have the diagnostic capacity to distinguish bovine tuberculosis from the more common form of human tuberculosis (DFID, 2005), because of a number of reasons including logistic, culture facilities and expertise. These difficulties have contributed to the general lack of epidemiological information on zoonotic disease in sub-Saharan Africa (Anon, 1963; Holden, 1999; Perry *et al.*, 2002).

The diagnosis of brucellosis is based on the clinical features and the results of laboratory. tests (FAO/WHO, 1985). Since many other diseases with similar clinical features occur commonly amongst the poor, the diagnosis of brucellosis in such communities depends on laboratory investigation. The definitive diagnosis of brucellosis could be made by culture and isolation of the causative organisms, but the procedure requires special media and it takes several weeks of inoculation and has low sensitivity in acute cases (Gotuzzo *et al.,* 1986; Christie, 1987; Araj *et al.,* 1990). The SAT is used in sero-prevalence studies, and has been used in sero-diagnosis of bovine brucellosis in Tanzania (Mahlau and Hammond 1962; Staack and Protz, 1973). However, this technique has two disadvantages: first, cross reactions occur with bacteria related to the genus *Brucella* such as *Afipia, Francissela, Vibrio* and *Yersinia* species and second, positive results occur in exposed populations and following cure (Goicochea *et al.,* 1996; Drancourt *et al.,* 1997).

Although more sensitive tests are available, SAT is still useful in providing data sufficient to differentiate active from inactive disease when other factors such as clinical history and follow-up sera are considered (Young, 1991). The use of agglutination reaction, as the sole technique for the diagnosis of brucellosis, should be considered carefully in endemic areas in the context of patients who are exposed repeatedly to *Brucella* or have a history of brucellosis (Ruiz-Mesa *et al.,* 2005). Other tests used for diagnosis of brucellosis include, the Complement Fixation Test (CFT) (Alton *et al.,* 1975), Fluoroscent Polarization Assay (FPA) (Nielsen *et al.,* 2000), ELISA (Lucero *et al.,* 1999),

radioimmunoassay (Parratt *et al.,* 1977), the indirect immunofluorescence assay

(Colmenero *et al.,* 1989), and the 2-mercaptoethanol test (Reddin *et al.,* 1965).

Several studies have been conducted to evaluate the sensitivity of agglutination tests as a

tool for diagnosis of brucellosis (Young, 1991; Oomen and Waghela 1974; Colmenero *et*

al., 1989). In a study conducted by Mert *et al.,* (2003), the sensitivity of SAT was found to

be 100% and in another study conducted by Memish *et al.,* (2002) the sensitivity of SAT

was found to be 96.65%. In Turkey, a study conducted by Ciftci *et al.,* (2005) showed the

sensitivity of the RBPT to be 100%, and 94.3% for STA. In Spain, Gall and Nielsen (2004)

demonstrated the overall sensitivity of the RBPT as 92.9%. In a few studies however, such

as that conducted by Sirmatel *et al.,* (2001), low sensitivities of standard tube agglutination

and Rose Bengal tests (83.7%, and 67.9% respectively) were obtained.

The specificity of the agglutination tests has been shown by many studies to be high. In a

study conducted in Spain by Gall and Nielsen (2004), the overall specificities of the RBPT

for groups of patients with history of brucellosis, regular exposure to brucellosis and those

treated for brucellosis were 94.3%, 91.7% and 76.9%, respectively, with positive likelihood

ratios of 16.5, 10.4 and 4.2. Memish *et al.,* (2002), and Mert *et al.,* (2003) demonstrated the

specificity of standard agglutination test to be 100%. The high specificity results of

agglutination tests are a good indicator for diagnosis of brucellosis where many diseases

with similar clinical features and those eliciting similar antigenic response co-exist.

However, as stated earlier in this chapter, the use of agglutination reaction, as the sole

technique for the diagnosis of brucellosis, should be considered carefully in endemic areas

where patients are exposed repeatedly to *Brucella* or have a history of brucellosis. A thorough clinical history needs be taken and at times follow-up sera should be considered.

Agreement between agglutination tests and other tests has also been studied in different parts of the world. Maichomo *et al.,* (1998), conducted a study on the agreement between the RBPT performed at three hospitals in Narok, Kenya. He was able to establish that the performance of the RBPT was good, but staff performing the tests needed more training as the agreement between the RBPT, the SAT and the CFT was poor. Oomen and Waghela (1974) and Morgan *et al.,* (1969) also established a good correlation between the RBPT and the SAT with low false positive and false negative rates. They concluded that the RBPT and the SAT were reliable tests for the diagnosis of human brucellosis especially in areas where laboratory facilities are not available.

The retrospective hospital study and the cross-sectional survey that were conducted in Arusha and Manyara regions between May 2002 and July 2003 provided an opportunity to know the types of tests used at the hospitals in Arusha and Manyara regions for diagnosis of brucellosis and to establish the prevalence of brucellosis in Arusha and Manyara regions using different tests (see chapter 3). In the retrospective hospital survey, hospitals were found to use different tests and during the cross-sectional survey it was established that prevalence of brucellosis was 4.8% based on the field RBPT, 6.4 % based on the RBPT performed at the SUA, and by using the c-ELISA test performed at the VLA the prevalence of brucellosis was 8.3% (see chapter 4). However, the performance of the tests used need to be evaluated so that we can know the validity of the results.

This study therefore intends to determine the validity of brucellosis diagnostic tools used at the hospitals in Arusha and Manyara regions and also to determine the validity of the RBPT as a diagnostic tool for brucellosis in the field and hospitals. The aim is to improve the diagnosis of brucellosis and allow prompt treatment. It is our hope that this will alleviate the burden caused by brucellosis to the people living in mainly rural areas of Tanzania.

7.3   Materials and methods

7.3.1   Study area

The study area and the hospitals involved with the study have been discussed in the second chapter of this work.

7.3.2   Study design and sampling

Serological data for evaluation of diagnostic tests were obtained from the cross-sectional study and the hospital clinical study. In the cross-sectional study, subjects for blood sampling were obtained by multi-stage cluster sampling discussed in chapter 2. In the clinical study, all patients presenting with clinical features suggestive of brucellosis (brucellosis suspects) were tested for brucellosis using antigen purchased by the hospital and the RBPT supplied by the project. Further analyses performed included the RBPT and the c-ELISA at the SUA and the c-ELISA test at the VLA.

Samples collected from suspects in hospitals were processed and tested for brucellosis at the hospitals using both the RBPT and local hospital tests in current use for diagnosis of brucellosis (Table 3. 5). The remaining aliquot was stored at $-20^{0}C$ for further analysis at the SUA and the VLA as described above. Details of the RBPT and the c-ELISA test conducted have been described in chapter 2 of this thesis.

7.3.3 Analysis

Samples collected and tested in the cross-sectional survey, at the laboratory in SUA and at hospitals were subjected to the c-ELISA test at the VLA. This test was taken as a gold standard. The test results were entered and analyzed in Excel spread sheets. Diagnostic sensitivities (Se), specificities (Sp), positive and negative predictive values (Ppv, Npv respectively) were calculated as described by Thrusfield (2001). The Youden's index (YI) was calculated as: Se +Sp – 1 (Youden, 1950). The accuracy (Index of validity) and likelihood ratios calculations based on a method described by Sackett *et al.,* (1991) as: Accuracy = (True positives+true negatives)/Total tested)*100, the positive likelihood ratio = (1-Se)/Sp and the negative likelihood ratio = Se/(1-Sp). Cohen's Kappa agreement test (Cohen, 1960) was used to determine the agreement between the tests. Epi info 6 (CDC, Antanta, Georgia) was used to calculated the 95% confidence intervals.

To examine the trend of Se and Sp at different cut-off points, and to calculate the best cut-off point, the Receiver Operating Characteristic (ROC) curve was drawn using Win Episcope software (CLIVE, Learning Technology Section, College of Medicine and

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Veterinary Medicine, University of Edinburgh). The cut-off point that provided the

maximum Se and Sp was determined by the largest area under the curve. The method

used to calculate the area under the curve was the one described by Hanley and McNeil

(1982).

7.4   Results

7.4.1 Evaluation of the RBPT as a screening test for brucellosis in the cross-
sectional survey

The diagnostic sensitivity and specificity of the RBPT were 39.4% (95% CI, 21.8%-61.1%)

and 98.8% (95% CI, 97.2%-99.8%) respectively. The positive and negative predictive values

were 76.9% (95% CI, 46%-93.8%) and 95.7% (95% CI, 92.8-97.5%) and the Youden's index

was 0.39 (Table 7.1).

Table 7. 1: Performance of the RBPT as a cross-sectional survey-screening test

	c-ELISA +ve	c-ELISA -ve	Total	Se	Sp	Ppv	Npv	YI
RBPT+ve	13	4	17	39.4	98.8	76.5	95.1	0.39
RBPT-ve	20	387	407					
95% C I				21.8-61.1	97.2-99.8	46.0-93.8	92.8-97.5	
Total	33	391	424					

7.4.2   The performance of the RBPT for diagnosis of brucellosis in a laboratory
setting

The performance of the RBPT as a laboratory test for diagnosis of brucellosis was

conducted through analyses at the laboratory in SUA. The principal investigator and

laboratory staff at the SUA were involved in the testing. The diagnostic Se and Sp of the RBPT at the SUA laboratory was 38.7% and 96.8% respectively (95% CI, 29.5%-48.7% and 95.8%-97.6% respectively) and the Youden's index was 0.36 (Table 7.2)

Table 7. 2: Performance of the RBPT as a laboratory test for brucellosis

	c-ELISA +ve	c-ELISA-ve	Total	Se	Sp	Ppv	Npv	YI
RBPT +ve	41	53	94	38.7	96.8	43.6	96.1	0.36
RBPT-ve	65	1591	1656					
95% C I				29.5-48.7	95.8-97.6	33.5-54.2	95-96.9	
Total	106	1644	1750					

7.4.3 Performance of the RBPT as a diagnostic test for brucellosis in hospital settings

The Rose Bengal antigen was supplied by the VLA to hospitals, and hospital laboratory staff were trained on how to test serum samples by the plate agglutination method (RBPT). The diagnostic Se and Sp of the test as performed in hospitals was 44.3% and 89.5% (95% CI, 34.4-54.8 and 87.8- 91 respectively), Youden's index was 0.34 (Table 7.3).

Table 7. 3: Performance of the RBPT in hospitals

	c-ELISA +ve	c-ELISA –ve	Total	Se	Sp	Ppv	Npv	YI
RBPT +ve	43	159	202	44.3	89.5	21.3	96.2	0.34
RBPT –ve	54	1356	1410					
95% C I				34.4-54.8	87.8-91.0	16-27.7	95.0-97.1	
Total	97	1515	1612					

7.4.4 Performance of antigens used in hospitals

All the antigens used at the hospitals were evaluated. The overall performance of the tests combined and of individual tests was performed. The overall diagnostic Se and Se of the combined tests were 47.7% and 82.1%, with Youden's index of 0.29 (Table 7.4).

Table 7. 4: Performance of antigens used in hospitals

Test	c-ELISA+ve	c-ELISA -ve	Total	Se	Sp	Ppv	Npv	YI
Hospital tests +ve	44	268	312	47.7	82.1	14.1	96.2	0.29
Hospital tests –ve	49	1227	1276					
95% C I				37-57.9	80-84	10.5-18.6	94.9-97.1	
Total	93	1495	1588					
Chronolab +ve	34	188	222	45.9	83.6	15.3	96	0.29
Chronolab –ve	40	957	997					
95% C I				34.4-57.9	81.3-85.7	11-20.9	94.5-97.1	
Total	74	1145	1219					
Murex +ve	1	30	31	50	56.5	3.2	97.5	0.07
Murex –ve	1	39	40					
95% C I				2.7-97.3	44.1-68.2	0.2-18.5	85.3-99.9	
Total	2	69	71					
Nairobi RBPT +ve	3	43	46	50	74.3	6.5	97.6	0.24
Nairobi RBPT –ve	3	124	127					
95% C I				13.9-86.1	66.8-80.6	1.7-18.9	92.7-99.4	
Total	6	167	173					
Eurocel A +ve	6	7	13	75	90	46.2	96.9	0.65
Eurocel A –ve	2	63	65					
95% C I				35.6-95.5	79.9-99.5	20.4-73.9	88.4-99.5	
Total	8	70	78					

7.4.5 Sensitivity and specificity of the RBPT at different cut-off points of the c-ELISA test percentage optical density.

The Se and Sp of RBPT conducted in the cross-sectional survey were calculated at different cut-off points of percentage of the c-ELISA test Optical Density (OD), so as to

examine the effect of cut-off point on Se and Sp (Table 7.5). Using Win Episcope 2(CLIVE, Learning Technology Section, College of Medicine and Veterinary Medicine, University of Edinburgh), a ROC curve (Figure 7.1) was drawn and the cut-off point with the best results of both Se and Sp was calculated by using the method by Hanley and McNeil (1982). The area under the ROC curve was calculated as 66.5% (95% CI, 65.0%-67.9%). At this cut-off point the Se and Sp of the RBPT in the cross-sectional survey were 37.14% and 98.97% respectively.

Table 7. 5: Se and Sp of the RBPT at different cut-off points of percentage of the c-ELISA test OD

Cut off point	Sensitivity	Specificity
30	64.7	98.5
40	57.1	98.8
50	50.0	98.9
60	39.4	98.8
70	34.2	98.9
80	23.6	98.9
90	15.8	99.4
100	4.5	98.6

Figure 7. 1: Receiver Operating Characteristic (ROC) curve for the values of Se and Sp at different cut-off points.

Class	Infected	Non-Infect.
30	17	407
40	21	403
50	26	398
60	33	391
70	38	386
80	55	369
90	95	329
100	71	353

		Lower	Upper Lim.	
Area (%):	66.480	65.020	67.940	✕ Close
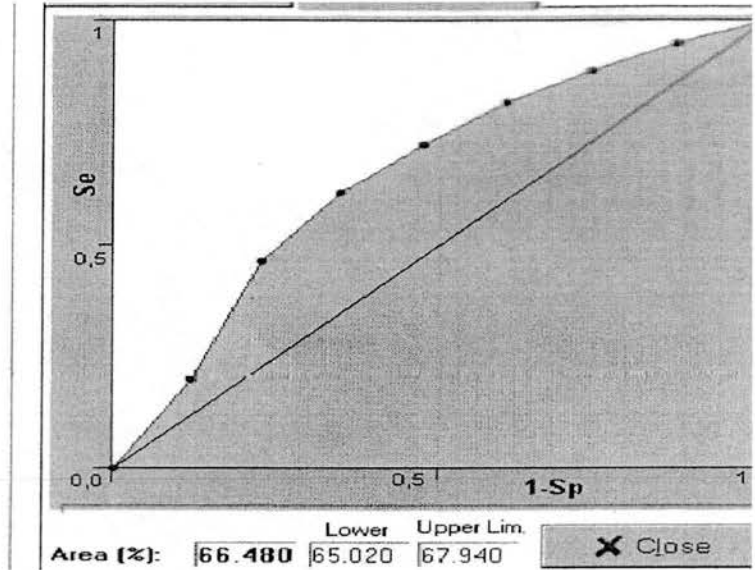
7.4.6 Accuracy (Index of validity), positive and negative likelihood ratios of the tests

The RBPT performed at the SUA laboratory had the highest accuracy and likelihood ratio of a positive test (0.95 and 69.50 respectively) compared to other tests, followed by the RBPT performed in the cross-sectional survey, and Eurocel A®. Murex® had the lowest accuracy and likelihood ratio of positive test (Table 7.6)

Table 7. 6: Accuracy, positive and negative test likelihood ratios of the tests

Test	Accuracy	Likelihood ratio Positive test	Likelihood ratio Negative test
Overall (Hospitals)	0.8	3.50	0.61
RBPT cross-sectional Survey	0.90	12.09	0.63
RBPT laboratory	0.95	69.50	0.59
RBPT hospital	0.86	4.22	0.60
Chronolab®	0.81	2.70	0.60
Murex®	0.56	1.10	0.80
Nairobi RBPT	0.70	1.95	0.67
Eurocel A®	0.88	7.50	0.28

7.4.7 Agreement of the tests

Cohen's Kappa agreement test (Cohen, 1960) was used to assess the agreement between the RBPT performed at the SUA and all the other tests (The RBPT performed in the field and all the tests performed at the hospitals) (Table 7.7).

Table 7. 7: Agreement between the different tests and the RBPT performed at the SUA laboratory

Test	Kappa agreement
Cross-sectional survey RBPT	0.56
Hospital RBPT	0.21
Chronolab®	0.12
Murex®	0.11
Nairobi RBPT	0.06
Eurocel A®	0.08

7.5   Discussion

The RBPT used at the hospital and in the field, and the hospital antigens, showed low diagnostic sensitivities. Based on the results, it is most likely that brucellosis is underdiagnosed in Arusha and Manyara regions. The RBPT supplied by the VLA had the diagnostic sensitivities of 39.4% in the cross-sectional survey, 38.7% at the SUA laboratory and 44.3% in hospitals. The different antigens used by the hospitals were no better with the most sensitive being Eurocel A® used by Wasso hospital in Ngorongoro district which gave a sensitivity of 75%. The results indicated that the antigens were actually poor in the diagnosis of brucellosis. This was supported by the low likelihood ratio of positive tests results, with the RBPT conducted at the SUA laboratory having the highest value (69.5) followed by the RBPT in cross-sectional survey (12.09).

The tests showed high specificities with the RBPT performed in the cross-sectional the highest (98.9%) followed by the RBPT performed at the SUA laboratory (96.8%) and Eurocel A® (90%). The other antigens used at the hospitals had specificities ranging from 56.5% (Murex®) to 83.5% (Chronolab®). The results were supported by the likelihood ratio of negative tests results which were below 1, with Eurocel A® having the lowest ratio (0.28).

There was poor agreement between the tests performed at different settings and the RBPT performed at the SUA. The RBPT performed in cross-sectional survey had the highest value of agreement (56.7%) and Eurocel A® the lowest (0.08%). Besides the fact that Eurocel A® had the highest sensitivity and specificity amongst the tests performed at the hospitals, there was a poor agreement between it and the RBPT performed at the SUA. The results indicated that in addition to the antigens themselves a local technical problem could have influenced the test results.

The results of the RBPT were rather unexpected and differed from the results of studies done elsewhere (Young, 1991; Sirmatel *et al.,* 2001; Mert *et al.,* 2003; Gall and Nielsen, 2004; Ciftci *et al.,* 2005) in which the sensitivity of the RBPT was observed to be high. The low sensitivities of the antigens in cross-sectional survey, hospitals and in laboratory settings make them unsuitable for use in the diagnosis of brucellosis in Tanzania. The poor agreement of the tests showed a possibility of shortfalls in sample handling or testing techniques. Several other factors might have contributed to the poor performance of the tests such as freeze, thaw cycle between the tests and the strain variation. On the other hand the c-ELISA test is not by itself 100% sensitive; this might also have an influence on the low sensitivities of the tests.

Changing the cut-off point of the c-ELISA test did not seem to have a profound influence on the diagnostic specificity. Lowering the cut-off point for example to 30, apart from decreasing the number of cases detected by 48.8%, it increased the sensitivity of the test to only 64.7% (64.3% increase) which is still low for a test to be considered valuable for brucellosis diagnosis. Increasing the cut-off point on the other hand, decreased the sensitivity of the test still further. At the cut-off point of the c-ELISA test OD of 66.7% (from ROC curve), the sensitivity of the RBPT decreased at the expense of specificity which increased by 0.01%. The results vindicated the cut-off point of 60% because other cut-off points did not give higher sensitivity and specificity results.

7.6   Conclusion

The sensitivities of the antigens evaluated were too low to support them as reliable tests for diagnosis of brucellosis in the study area. A number of factors could have contributed to the

poor performance of the tests. These include *Brucella* strain variation in the study area, the storage of the samples during transportation and errors during testing. There is a need to carry out more variations of the tests with improved conditions and a study to establish the types of strains common in the study area. This might help in improvement or modification of the antigens used.

Staff involved should take extra care in handling specimens for testing so as to maintain their optimal ability to form agglutination during testing. The staff should try to abide by tests' procedures such as use of clear background, stick to test-specific times during testing and proper storage of reagents. If conditions allow, there is a need to conduct regular quality assurance surveys of diagnostic tools for brucellosis. This will ensure that proper laboratory procedures such as proper storage and sample handling are followed.

The number of antigens used for testing brucellosis in the study area, is an issue of particular concern. There is a need to monitor and standardise the protocols and guidelines for diagnosis of brucellosis in Tanzania if the Ministry of Health is to be serious with brucellosis case detection. Advanced tests such as the c-ELISA test are not cost-effective for conducting routine brucellosis testing in the study area. It is therefore unlikely that hospitals in the study area could afford acquisition of tests such as the c-ELISA test for routine testing of brucellosis. It is however possible to establish zonal laboratories in areas where brucellosis is thought to be common, where samples of difficult or chronic cases could be analysed, with such confirmatory tests as the c-ELISA test. This would also minimize the problems encountered during shipment of samples over a long distance.

8.   Estimating the burden caused by brucellosis in Tanzania

8.1 Summary

Objectives: To assess the burden of brucellosis in Tanzania in terms of Disability Adjusted Life Years (DALY) and direct and indirect costs to the health provider and to the patient.

Methodology: The study applied the World Health Organization (WHO) and the World Bank (WB) recommended DALY to estimate the burden of disease caused by brucellosis in Tanzania. Data for DALY estimations were obtained from the cross-sectional survey and cases established during the prospective hospital survey in Arusha and Manyara regions. Socio-demographic characteristics, economic status, clinical history, and data on costs of treatment were obtained from the cases by using questionnaires administered by the principal investigator of the study and from hospital records. The results obtained were extrapolated to the whole country and compared to DALY estimates caused by other diseases.

Results: By using disability weights of 0.2, discounting and duration of brucellosis of 4.5 years, 3,645 – 3,709 DALY burden were estimated to be caused by brucellosis based on hospital data, and by using disability weight of 0.2, discounting and duration of one year, 92,080 – 121,550 worth of DALY were estimated to be caused by brucellosis based on data from the cross-sectional survey. The majority of cases continued to show clinical features of brucellosis for a period of over two years, and out of these, five days were spent as inpatients. During the period of two years, the productive force of the cases was lowered. Each household used a total of US $ 90.65 (92, 826 TShs.) to care for a single case of brucellosis per year and each health provider used a total of US $ 858 (878,592 TShs.) per year to care for cases of brucellosis.

Discussion and conclusion: Brucellosis contributes to poverty and suffering particularly to the poor in the rural areas of Tanzania and yet it is neglected. The burden caused by brucellosis based on hospital data was found to be more than thirty times less than the burden estimated from cross-sectional data. It is therefore important to consider calculating DALY using data based on the general community, particularly in developing countries where few cases present to hospital and when the disease runs a chronic course. Data collecting and recording systems in Tanzania should be updated so that diseases such as brucellosis can be notified at the district, regional and national levels for a better epidemiological picture of diseases that would potentially be under-reported and neglected. More attention needs to be directed towards control of brucellosis in Tanzania. However, the control measures should also take into account the cost effectiveness of intervention strategy as Tanzania is a poor country that requires care in the allocation of resources.

8.2 Introduction

The DALY is a quantitative indicator of burden of disease that reflects the total amount of life lost, to all causes, from premature mortality or from some degree of disability during a period of time (World Bank, 1993). Other metrics for burden of disease include analyses of direct monetary costs of medical care, and the indirect costs related to lost wages and productivity (USA Department of Health, 2000; Murray, 2002). The DALY was designed as a measurement unit for the Global Burden of Disease (GBD) study to be used for two tasks: first, as a unit for measuring the magnitude of premature death and non-fatal health outcomes attributable to causes, including diseases and injuries, poor water supply, tobacco use or socio-economic inequality (Murray and Lopez, 1996); and second, as a measure for cost-effectiveness analyses of interventions that could reduce the burden of the causes and socio-economic determinants listed above. The development of DALY was intended to make the ethical dimensions of quantifying health more transparent (Murray and Lopez, 1996).

The World Health Organization (WHO) has consistently indicated the continent of Africa as the place that deserves much more attention for future health investment (WHO, 2002). The majority of the world's burden of disease is in sub-Saharan Africa (Figure 8.1). In 2001 for example, an estimated 24.4% of the global burden of disease was borne by Africa, home to only 10.7% of the global population (WHO, 2002). The greater part of the DALY for sub-Saharan Africa is caused by high mortality in early childhood from a number of infectious diseases. It is estimated that malaria alone

contributes to a loss of 36.8 million DALY, of which 34 million are in Africa alone

(WHO, 2002).

Figure 8. 1: DALY per 1000 population by region broken down into years lost due to premature death (YLL) and years lived with disability (YLD) rates in 1990
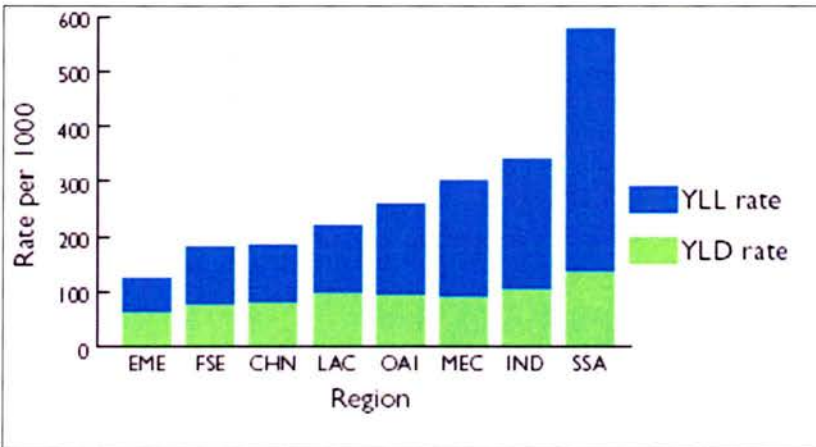
Graph by Burden of Disease unit, Harvard University

EME- Established Market Economies
FSE-Formerly Socialist Economies of Europe
CHN-China
LAC-Latin America and the Caribbean.
OAI -Other Asia and Islands
MEC-Middle Eastern Crescent.
IND-India
SSA-Sub-Saharan Africa.

Many zoonoses occur in Africa, in particular, the sub-Saharan area. However, it has been

difficult to appreciate the size of the burden posed by the zoonotic diseases in sub-

Saharan African countries at a continental and national level (Cattand *et al.*, 2001). The

number of cases reported to the WHO by the individual countries either represents a

small proportion of cases or cases are not reported at all. In Zimbabwe for example, one

of the very few national burden of disease estimations was conducted by Chapman *et al.*,

(2006). Results showed that none of the zoonotic diseases featured as the cause of disease burden in Zimbabwe. This is provides evidence that zoonotic diseases are under-diagnosed or neglected and hence not accounted as among the major cause of disease burden in the continent.

Many factors have been put forward as contributing to this general lack of data on zoonoses. These include diagnostic difficulties, limited surveillance coverage, geographical distribution of those mostly affected and the general lack of knowledge of the threat posed by zoonoses (WHO,1998b; Cleaveland *et al.,* 2002; Cattand *et al.,* 2001). Brucellosis is a well known disease in many countries, but official figures produced by individual countries and international institutions do not fully reflect the actual number of people infected each year, and the true incidence has been estimated to be between 10 and 25 times higher than reported figures indicate (WHO, 1997).

Disease burden and economic growth are intimately related. Zoonoses have a significant impact on the economic development of the poor people in developing countries (Perry *et al.,* 2001; Shaw, 2004). The losses caused by zoonotic diseases are both directly due to morbidity, mortality to both humans and livestock, and to reduction of fertility, milk yield and ability for animals to work as traction animals, and indirectly such as due to the costs of ineffective control measures, limiting production opportunities, and influencing the choices made by livestock keepers and farmers (Perry *et al.,* 2001; Shaw, 2004). *Brucella* is one of the world's major zoonotic pathogens, and is responsible for enormous economic losses as well as considerable human morbidity in endemic areas (Boschiroli

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et al., 2001) with a high prevalence in the Mediterranean countries (Lulu *et al.*, 1988;

WHO, 1990). It is also present in Asia, sub-Saharan Africa and Latin America (Abu

Shaqra, 2000; Domingo, 2000; Mikolon *et al.*, 1998). Clinical manifestations of

brucellosis in humans include joint pain, backache, headache, fatigue and loss of appetite

(see chapter 5). Other common features include drenching sweats, spleen and liver

enlargement in advanced stage (Corbel, 1989b; Madkour, 1989). The young and middle-

aged adults who are the most productive force, are the ones mostly affected, with a low

incidence among infants and elderly patients (Lulu *et al.*, 1988) see chapter 2 and chapter

4. Transmission is through direct contact with infected animals or their products.

Unpasteurized milk and processed dairy foods from infected animals are the major

source of infection for the general population (Corbel, 1989a). Acute brucellosis may

occur after an incubation period of 2 –3 weeks, and chronic form up to one or two years,

during which the patient may be severely debilitated (Young, 1995b). The case-fatality

rate of untreated brucellosis is 2% or less and is caused mainly by endocarditis (Jacobs *et*

al., 1990).

Plate 8. 1: Weak and grossly emaciated brucellosis case (Picture by Tim Fison)

The benefits of zoonotic diseases control need to account for dual benefits to public health and livestock sectors. A study conducted in Mongolia by Roth *et al.*,(2003) to estimate the economic benefits, cost-effectiveness, and distribution of benefit of improving human health, found that a total of 49,027 DALY could be averted by mass vaccination of animals against brucellosis. Estimated intervention costs were US$ 8.3 million, and the overall benefit was US$ 26.6 million. If the costs of vaccination of livestock against brucellosis were allocated to all sectors in proportion to the benefits, the intervention might be profitable and cost effective for the agricultural and health sectors.

Plate 8. 2: In chronic form, brucellosis manifests as a severe debilitating condition

Brucellosis was first reported in Tanzania in livestock in the then Tanganyika in 1928. In humans brucellosis was first reported in 1935 by Evans (1935). Livestock surveys carried out in Tanzania at different times have shown prevalence of brucellosis varying from 2%-97% (Mahlau, 1967; Kitaly, 1984; Minga and Balemba, 1990; Jiwa *et al.,* 1996; Weinhaulp *et al.,* 2000). However, studies to assess the burden caused by brucellosis to humans in-terms of DALY loss and the costs of treatment have not been conducted. The current study aims to estimate the burden of brucellosis in Tanzania in terms of DALY losses and costs of treatment to both the patient and the health provider. This was the first study of its kind to be conducted in Tanzania. It is hoped that the estimation of the burden caused by brucellosis will stimulate the estimation of the burden

of other diseases using DALY instead of using only mortality and morbidity. This will create an enabling environment for easier health policy formulation.

8.3   Methodology

8.3.1 Study area

Detailed information on the study area and hospitals involved with the study, have been described in the second chapter of this report. A brief account of methodology applied specifically to this chapter is described.

8.3.2 Data collection

Data for DALY estimations were obtained from the cross-sectional survey and the prospective hospital survey in Arusha and Manyara regions. Methodology used for case recruitment both in the cross- sectional and in the prospective hospital survey have been described in sections 2.4.1 and 2.5.1 respectively of this thesis. Socio-demographic characteristics, economic status, clinical history, and data on cost of treatment were obtained from the cases by using open-ended questionnaires administered by the principal investigator of the study (Appendix III). Data on human population size in the study areas were obtained from national population and housing census conducted in 2002 (Tanzania population and housing census, 2002) and from district and regional councils. Data on standard life expectancy was obtained from Standard Model West level 26 (Murray, 1994).

In Tanzania both the patient and the health provider share the costs of treatment in what is termed as the "cost sharing system". Extra costs (indirect costs) are however incurred by patients for transport, meals and at times accommodation for the patient and any accompanying person/s, during the course of treatment. In the current study, data on patients' direct and indirect costs of treatment were obtained from both case follow-up and hospital records.

8.3.3 DALY parameters

8.3.3.1 Disability weights (D):

Disability weight (D) is the degree of incapacity. D has six classes defined with weight 0 as perfect health and 1 as death (Murray, 1994) (Table 8.1). DALY was calculated using class 2 (weight 0.2) which has also been used by other studies (Roth *et al.*, 2003).

Table 8. 1: Definition of disability weighting used in DALY calculation

Class	Description	Weight
1	Limited ability to perform at least one activity in of the following areas: Recreation, education, procreation or occupation	0.096
2	Limited ability to perform most activities in one of the following areas: Recreation, education, procreation or occupation	0.220
3	Limited ability to perform activities in two or more of the following: Recreation, education, procreation or occupation	0.400
4	Limited ability to perform most activities in all of the following areas: Preparation, shopping or housework	0.600
5	Needs assistance with instrumental activities of daily living such as meal Preparation, shopping or housework	0.810
6	Needs assistance with instrumental activities of daily living such as eating, personal hygiene or toilet use	0.920

8.3.3.2 Age weights:

This is the parameter that shows the relative importance of healthy life at different ages. The parameter rises up to 25 years and then starts to decline (Figure 8.2). In the present study DALY was calculated with and without age weighting function. Age weighting function was calculated from the formula: Age weighting = $(Cxe^{-\beta})$.

Where:

- C = Constant equal to 0.16243.
- β = Constant equal to 0.04.
- x = Age.
- e = Constant equal to 2.71.

Figure 8. 2: Variation of relative weight (Relative importance of life) with age
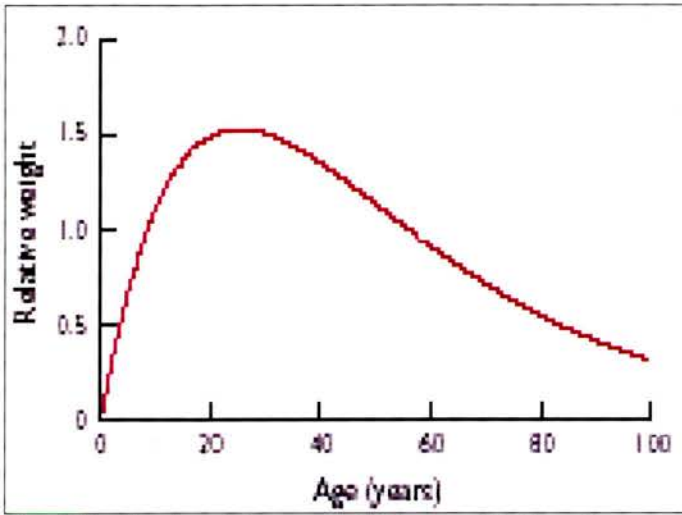
Graph by Burden of Disease unit, Harvard University

8.3.3.3 Time preference:

This is the value of health gains today compared to the value attached to the health gains in the future. Using the discount rate of 3%, the discounting function was calculated as: $(e^{-r(x-a)})$.

Where:

- r = Discount rate, fixed at 0.03
- x = Age.
- e = Constant equal to 2.71
- a = Onset year.

8.3.3.4 Duration of time lost due to a death at each age or Years of Life Lost due to premature death (YLL):

This is the years of life lost due to premature death. YLL is calculated as $(Cxe^{-\beta})(e^{-r(x-a)})$.

Standard life expectancy at birth is taken as 82 for females and 80 for males (according to model life-table West level 26) (Murray, 1994) based on Japanese female and male

life expectancies respectively. The current study used the model life-table West level 26 to allow comparisons with other studies on DALY conducted (Murray and Lopez, 1996).

8.3.3.5 Duration of brucellosis:

The duration of brucellosis clinical features was taken as 4.5 years. This was the time established by (Beklemischew, 1968) and has been used in other studies (Roth *et al.,* 2003).

8.3.3.6 Years of Life Lost due to disability (YLD)

This is the years of life lost due to a non-fatal condition, it is calculated from the formula, $YLD = I * D * L$

Where:

- I = number of incident cases
- D = disability weight
- L = average duration of the case until remission or death (years).

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8.3.4 Assumptions made during calculation of DALY for the whole of Tanzania

To extrapolate the burden of brucellosis for the whole of Tanzania from data collected in Arusha and Manyara regions, the following assumptions were made:

- Brucellosis is common in all regions with people keeping livestock. Regions such as Mtwara, Lindi and the islands of Zanzibar and Pemba where livestock are not commonly kept were excluded from the study.

- Brucellosis is common in rural areas that make up 75% of the Tanzanian population (World Bank, 2002). In rural areas there is a close relationship between humans and livestock and activities such as assisting parturition are common. The total population involved in DALY calculation was therefore 75% of the total population of Tanzania excluding Mtwara, Lindi and the islands of Zanzibar and Pemba. This was calculated as 24,392,862 people.

- Although in the current study brucellosis was found to be more common in pastoral than in agropastoral areas, the pastoral community compose a very small proportion of livestock keepers in the whole of Tanzania. The incidence of brucellosis was therefore assumed to be the same in both pastoral and agropastoral areas.

- As the number of brucellosis cases reporting to hospitals represent only a fraction of those suffering from the disease in the community, DALY were calculated using data obtained during cross-sectional and during hospital surveys for comparison purposes.

8.3.5 Calculation of DALY

The total DALY score was calculated by using the formula:

$DALY(x) = (D)(Cxe^{-\beta})(e^{-r(x-a)}) + (Cxe^{-\beta})(e^{-r(x-a)})$, which is equal to YLD+ YLL. The number of incidence cases of brucellosis established and deaths due to brucellosis in Arusha and Manyara regions were extrapolated to the whole of Tanzania based on assumptions listed on section 8.3.4.

8.3.6 Estimating the burden of brucellosis in terms of direct and indirect costs to the patient/household and to the health provider

The costs of treatment were taken as the average of the total cost incurred by all the patients. In the current study it was evident that some brucellosis patients attended traditional healers prior to going to hospital and some attended several different hospitals for investigation and treatment and were prescribed a number of drugs. These costs were not included in the calculations, as it was difficult to establish the exact costs that cases incurred. All the mean costs, which were taken as costs per case, were calculated as the total costs divided by the number of cases. The costs incurred by each hospital were calculated as the total costs incurred by all hospitals divided by the number of hospitals.

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Plate 8. 3: Health facilities are overstretched to accommodate the number of patients requiring investigation and treatment for brucellosis

The annual income per household of agropastoral families was calculated from annual sales of crops (if it is assumed none was left for household consumption) divided by the number of households. One bag of maize was 12,000 TShs., of beans 40,000 TShs., of millet 15,000 TShs. and of sunflower 40,000 (data from markets in the study area as of June 2003). The Gross National Income (GNI) of Tanzania is US $ 340 (World Bank, 2006).

8.4   Results

8.4.1 Common febrile conditions in the study area

Of 1,153 patients who were seen by practitioners in the hospitals, 304 (26.37%) were

diagnosed and treated for malaria and 192 (16.65%) treated for joint pain categorised

as arthritis. Pain such as back and waist pain were also included in this category.

Typhoid fever ranked third with 199 (17.26%) patients while 166 (14.40%) patients

were treated for brucellosis. Detailed information about other febrile /flu-like

conditions have been described in chapter 5 of this work.

8.4.2 Brucellosis cases and incidences for DALY calculation

Of 428 individuals who were tested with the c-ELISA test at the VLA during the

cross-sectional survey, 33 tested positive. These were extrapolated to 467,340

brucellosis cases in the whole of Tanzania. As no fatality was recorded during the

cross-sectional survey, YLL was not calculated. During the hospital survey, 98

brucellosis cases were established and out of these five cases died of brucellosis.

All five deaths were males of greater than 50 years of age. This was extrapolated to

2,721 cases and 139 male deaths per annum due to brucellosis in the whole of

Tanzania.

8.4.3 YLL, YLD and DALY for Tanzania

By using disability weights of 0.2, discounting and duration of brucellosis of 4.5 years, 3,645 – 3,709 DALY burden were estimated to be caused by brucellosis based on hospital data, and by using disability weight of 0.2, discounting and duration of one year, 92,080 – 121,550 worth of DALY were estimated to be caused by brucelloasis based on data from cross-sectional survey. The summary of parameters used, calculation involved and results for YLL, YLD and total DALY are in tables 8.2-8.8.

Table 8. 2: YLL for males in hospital survey

Age groups	Population	Deaths	Av. age at death	YLLs	YLLa
0-4	2,007,569.0	0.0	0.0	0.0	0.0
5-14	3,408,809.0	0.0	0.0	0.0	0.0
15-29	3,172,484.0	0.0	0.0	0.0	0.0
30-44	1,830,922.0	0.0	0.0	0.0	0.0
45-59	848,621.0	28.0	52.6	537.0	436.0
60-69	355,681.0	0.0	0.0	0.0	0.0
70-79	207,680.0	83.0	75.8	706.0	380.0
80+	103,840.0	28.0	89.0	103.0	41.0
Total	**11,935,606.0**	**139.0**	**73.8**	**1,345.0**	**857.0**

YLLs – Years of Life Lost due to premature death without age weighting

YLLa - Years of Life Lost due to premature death with age weighting

Table 8. 3: YLD for females in hospital survey

Age Groups	Population	Incidence	Age at onset	Duration (years)	Disability Weight	YLDs	YLLa
0-4	2,006,864.00	0.00	2.50	4.5	0.2	0.00	0.0
5-14	3,370,934.00	56.00	10.00	4.5	0.2	47.15	58.0
15-29	3,587,690.00	361.00	22.50	4.5	0.2	303.92	463.0
30-44	1,884,782.00	583.00	37.50	4.5	0.2	490.82	660.0
45-59	893,185.00	194.00	52.50	4.5	0.2	163.33	166.0
60-69	383,684.00	28.00	65.00	4.5	0.2	23.57	18.0
70-79	215,510.00	28.00	75.00	4.5	0.2	23.57	14.0
80-	114,607.00	0.00	85.00	4.5	0.2	0.00	0.0
Total	**12,457,256.00**	**1,250.00**	**35.72**	**4.5**	**0.2**	**1,052.37**	**1,379.0**

YLDs – Years Lived with Disability without age weighting

YLDa – Years Lived Disability with age weighting

Table 8. 4: YLD for males in hospital survey

Age Groups	Population	Incidence	Age at onset	Duration (years)	Disability weight	YLDs	YLDa
0-4	2,007,569.00	0.00	2.50	4.50	0.2	0.00	0.0
5-14	3,408,809.00	250.00	10.00	4.50	0.2	210.47	260.0
15-29	3,172,484.00	333.00	22.50	4.50	0.2	280.35	427.0
30-44	1,830,922.00	389.00	37.50	4.50	0.2	327.50	440.0
45-59	848,621.00	250.00	52.50	4.50	0.2	210.47	214.0
60-69	355,681.00	83.00	65.00	4.50	0.2	69.88	53.0
70-79	207,680.00	139.00	75.00	4.50	0.2	117.02	68.0
80+	103,840.00	28.00	85.00	4.50	0.2	23.57	10.0
Total	11,935,606.00	1,472.00	37.98	4.50	0.2	1,239.27	1,473.0

YLDs – Years Lived with Disability without age weighting

YLDa – Years Lived Disability with age weighting

Table 8. 5: Total DALY in hospital survey

Age groups	Males			Females			All persons		
	Population	DALYs	DALYa	Population	DALYs	DALYa	Population	DALYs	DALYa
0-4	2,007,569.00	0.00	0.00	2,006,864.00	0.00	0.00	4,014,433.00	0.00	0.00
5-14	3,408,809.00	210.47	260.00	3,370,934.00	47.15	58.00	6,779,743.00	257.62	318.54
15-29	3,172,484.00	280.35	427.00	3,587,690.00	303.92	463.00	6,760,174.00	584.27	889.64
30-44	1,830,922.00	327.50	440.00	1,884,782.00	490.82	660.00	3,715,704.00	818.32	1,100.25
45-59	848,621.00	747.18	650.00	893,185.00	163.33	166.00	1,741,806.00	910.50	816.04
60-69	355,681.00	69.88	53.00	383,684.00	23.57	18.00	739,365.00	93.45	70.84
70-79	207,680.00	822.68	448.00	215,510.00	23.57	14.00	423,190.00	846.25	462.10
80+	103,840.00	126.20	51.00	114,607.00	0.00	0.00	218,447.00	126.20	51.20
Total	11.935,606.00	2,584.25	2,330.00	12,457,256.00	1,052.37	1,379.00	24,392,862.00	3,644.62	3,708.62

DALYa – DALY with age weighting

DALYs – DALY without age weighting

Table 8. 6: YLD for males in cross-sectional survey

Age groups	Population	Incidence	Age at onset	Duration (Years)	Disability Weight	YLDs	YLDa
0-4	2,007,569.00	0.00	2.50	1.00	0.20	0.00	0.00
5-14	3,408,809.00	14,162.00	10.00	1.00	0.20	2,790.34	3,190.49
15-29	3,172,484.00	127,456.00	22.50	1.00	0.20	25,112.63	38,160.65
30-44	1,830,922.00	42,485.00	37.50	1.00	0.20	8,370.81	11,534.90
45-59	848,621.00	42,485.00	52.50	1.00	0.20	8,370.81	8,829.75
60-69	355,681.00	0.00	65.00	1.00	0.20	0.00	0.00
70-79	207,680.00	0.00	75.00	1.00	0.20	0.00	0.00
80+	103,840.00	14,162.00	85.00	1.00	0.20	2,790.34	1,294.08
Total	11,935,606.00	240,750.00	33.38	1.00	0.20	47,434.92	63,009.87

YLDs – YLD without age weighting

YLDa – YLD with age weighting

Table 8. 7:YLD for females in cross-sectional survey

	Population	Incidence	Age at onset	Duration (years)	Disability Weight	YLDs	YLDa
0-4	2,006,864.00	0.00	2.50	1.00	0.20	0.00	0.00
5-14	3,370,934.00	14,162.00	10.00	1.00	0.20	2,790.34	3,190.49
15-29	3,587,690.00	99,133.00	22.50	1.00	0.20	19,532.15	29,680.68
30-44	1,884,782.00	70,809.00	37.50	1.00	0.20	13,951.48	19,225.01
45-59	893,185.00	14,162.00	52.50	1.00	0.20	2,790.34	2,943.32
60-69	383,684.00	14,162.00	65.00	1.00	0.20	2,790.34	2,206.30
70-79	215,510.00	0.00	75.00	1.00	0.20	0.00	0.00
80+	114,607.00	14,162.00	85.00	1.00	0.20	2,790.34	1,294.08
Total	12,457,256.00	226,590.00	34.84	1.00	0.20	44,644.98	58,539.88

YLDs – YLD without age weighting

YLDa – YLD with age weighting

Table 8. 8: Total DALY in cross-sectional survey

Age groups	Males			Females			All persons		
	Population	DALYs	DALYa	Population	DALYs	DALYa	Population	DALYs	DALYa
0-14	5,416,378.00	2,790.34	3,190.49	5,377,798.00	2,790.34	3,190.49	10,794,176.00	5,580.67	6,380.98
15-29	3,172,484.00	25,112.63	38,160.65	3,587,690.00	19,532.15	29,680.68	6,760,174.00	44,644.78	67,841.33
30-44	1,830,922.00	8,370.81	11,534.90	1,884,782.00	13,951.48	19,225.01	3,715,704.00	22,322.29	30,759.91
45-59	848,621.00	8,370.81	8,829.75	893,185.00	2,790.34	2,943.32	1,741,806.00	11,161.15	11,773.06
60-79	563,361.00	0.00	0.00	599,194.00	2,790.34	2,206.30	1,162,555.00	2,790.34	2,206.30
80+	103,840.00	2,790.34	1,294.08	114,607.00	2,790.34	1,294.08	218,447.00	5,580.67	2,588.17
Total	11,935,606.00	47,434.92	63,009.87	12,457,256.00	44,644.98	58,539.88	24,392,862.00	92,079.90	121,549.75

DALYs - DALY without age weighting

DALYa - DALY with age weighting

8.4.4 Burden of brucellosis in terms of direct and indirect costs to the patient and to the health provider

Data on household expenditures for brucellosis cases, and clinical history were available for 41 brucellosis cases. All the cases used a total of US $ 725.68 (743,100 TShs.)[†‡] for transport, investigation and treatment, an average of US $ 17.70 (18, 124 TShs.) per case per single presentation to hospital. Most patients had to get food from nearby hotels, or alternatively they bought cooking ingredients from nearby shops instead of getting these from home, thus each patient was estimated to use half a dollar for meals everyday.

Extra costs for transport of accompanying persons amounted to US $ 100.91 (102,319 TShs.), an average of US $ 2.26 per person/household (2,496 TShs.). Each household therefore incurred a total of US $ 22.66 (23,203 TShs.) for treatment and transport of a single case and other costs required by a person accompanying a case to hospital. As on average cases presented to hospitals four times in a year, the total costs incurred by each household to care for a single case of brucellosis amounted to US $ 90.65 (92, 826 TShs.). The costs for meals for persons accompanying the patients were not included as most of them spent nights within hospital premises and some with relatives or friends living close to the hospitals. The breakdown of the estimates is shown in Table 8.9.

[†] The exchange rate was US $ 1 for 1024 Tanzanian shillings
[‡] US $ = United States Dollar

Table 8. 9: Direct and indirect costs incurred by brucellosis cases in US $

	Investigations, drugs And transport	Meals	Accompanying Person	Total in US $ single presentation	Total in US $ four presentations
Costs for all cases	725.68	102.50	100.91	929.09	**3716.36**
Costs per case	17.68	2.50	2.46	22.66	**90.64**

Health providers incurred the costs of bedding, electricity, water, salaries for staff and a part of medication for patients. This was estimated at US $ 3.5 per day per patient in a rural setting (in Uganda it was US $ 2.5; Odiit, 2003)[§]. For all 98 cases that were diagnosed during the course of study, health facilities used a total of US $ 1,715 (1,756,160 TShs.). If the cost was to be shared equally among the eight hospitals, each hospital used US $ 214.50 (219,648 TShs.) per year to cater for brucellosis cases alone. As it was indicated earlier in the analysis, these were the costs of a single presentation to hospital. As cases attended to hospitals on four occasions within a year, each hospital used a total of US $ 858 (878,592 TShs.) per year to cater for brucellosis cases alone (Table 8.10).

[§] Tariffs are higher in Tanzania than in Uganda, estimated costs in Tanzania were made higher than in Uganda by US $ 1.

Table 8. 10: Costs incurred by health provider

Total costs by all hospitals per year for single presentation	Total costs per hospital per year, single presentation	Total costs per hospital per year, four presentations
US $ 1,715	US $ 214.50	US $ 858

In total, all brucellosis cases gave a history of symptoms of 29,602 days (722 days per case).

All the cases gave a history of limited daily work performance such as farming, cooking and

attending to animals while they were sick. Out of these days, a total of 204 days were spent in

hospital as inpatients (5 days per patient).

Plate 8. 4: Most of brucellosis cases are poor farmers in rural areas.

The mean expenditure per annum for school, health (including of brucellosis), food and other requirements was US $ 394.48 (403,951.50 TShs.) and the annual income of agropastoral families generated from sales of crops was estimated at US $ 363 (371,354 TShs.) per household, the Gross National Product (GNI) of Tanzania is US $ 340 (World Bank, 2006)

8.5   Discussion

Africa has the largest share of the world's burden of diseases (WHO, 2002). Estimation of burden

caused by diseases is therefore an essential strategy toward rational health planning. Prioritization of

different interventions, to a large extent, depends on the burden of the disease and cost effectiveness

of the intervention strategies, both of which can be estimated by using DALY (World Bank, 1993;

Murray *et al.*, 2002; Murray and Lopez 1996). The present study established that the disease burden

caused by brucellosis in Tanzania is enormous and yet it is neglected. Brucellosis contributes to

poverty and suffering particularly to members of animal keeping communities in the rural Tanzania.

Based on hospital data 3,645 – 3,709 DALY burden were estimated to be caused by brucellosis and

based on cross-sectional data 92,080 – 121,550 worth of DALY were estimated to be caused by

brucelloasis. In poor countries where health services are not easily accessible to many people, a

significant number of diseased individuals are likely to be in the community. As brucellosis cases

presenting to hospital represent just a fraction of real number of cases in the community it is

important to consider calculating DALY using data that is based on all cases that are in the

community, particularly for diseases that take a chronic course like brucellosis.

Data on DALY parameters for other diseases show that zoonotic tuberculosis caused by

Mycobacteria bovis caused 9,739 DALY loss, rabies 42,669, *Mycobacteria tuberculosis* (due to

human to human transmission) resulted in 730,891 DALY burden in the year 2000 (Coleman *et al.,*

2004). Data on the burden caused by other diseases in terms of DALY was scarcely available making

it difficult to make comparisons with many other diseases in Tanzania. The differences in rabies,

brucellosis and tuberculosis DALY values is attributed to the incidence/prevalence of the diseases,

fatality rate, the disability caused (presented as disability weight) and the age mostly affected. Other

factors that may attribute to the differences in DALY values include the methodology involved and the sample studied. Younger ages carry more YLL and YLD and hence more DALY than adults (Murray and Lopez, 1994). In Soroti, Uganda, DALY losses due to *T.b. Rhodesiense* sleeping sickness was estimated at 124 in 1999 (Odiit *et al.*, 2005). The DALY results were low because the sampling frame comprised of only a few health facilities in one area. There was a need to extrapolate the results to the country level.

On average brucellosis cases continued to have clinical features for a period of over two years, and of these, five days were spent as inpatients. This period is two and a half years less than the period observed in Russia by Beklemischew (1968) and which has been used in other studies (Roth *et al.*, 2003). During the period of sickness, cases gave a history of limited capacity to perform farm and home-based works and the employed recorded several days of absences. Students, who contributed 12% of all cases (see chapter 5), had irregular school attendances which could have an impact on their academic performances. Cases visited health facilities about 4 times within a year of illness instead of participating in production activities and it was even worse when the case was the household bread-winner.

Every case used about US $ 90.6 (92,826 TShs.) in a year to treat brucellosis. This amount is a heavy burden to poor extended households that have many other issues to deal with, and whose incomes depend on subsistence farming. The GNI of Tanzania is US $ 340 (World Bank, 2006) and for agropastoral families the annual earnings from crops selling was US $ 363. Using US $ 91 to treat a single case of brucellosis per year in an area with a burden of many other diseases, worsens the economies of the households in the study area still further. The non-specific manifestations of

brucellosis and their resemblance to other endemic diseases, such as malaria and typhoid in the study area made early diagnosis of brucellosis difficult. Extra costs for investigations and treatments were incurred by the cases and health providers to rule out other diseases that present like brucellosis. In addition to drugs for treating malaria, typhoid, and brucellosis, pain killers such as paracetamol, Ibuprofen, diclofenac and vitamins supplements were also found to be prescribed. Each hospital used a total of US $ 858 (878,592 TShs.) per year to cater for brucellosis cases which is a heavy burden to the already overstretched and under-funded health provider.

8.6   Conclusion

Brucellosis contributes to poverty and great suffering to people in Tanzania but yet it is underestimated and ignored. The study challenges public health specialists, in particular those involved with health planning, to take great care when estimating the burden caused by diseases. It is likely that some diseases are underestimated and neglected due to the nature of their clinical features and accessibility to healthcare for the people most affected. It is therefore important to consider estimating the DALY burden caused by diseases using data collected from the community rather than data from health facilities alone.

Brucellosis causes a significant loss of resources to those affected, their households and health providers hence still further worsening the economies of poor people and increasing the burden on the already underfunded health sector. Due attention needs to be directed towards control of brucellosis with appropriate intervention strategies focused on the areas mostly affected by the disease. This should include among other strategies, health education on ways brucellosis is

transmitted and improved diagnosis. However, any control measures implemented should also take into account their cost effectiveness as Tanzania is a poor country that requires great care in allocation of resources. Data collecting and recording systems in Tanzania should bé updated so that diseases such as brucellosis can be notified at the district, regional and national levels for better epidemiological information on diseases that would potentially be under-reported and neglected.

9. Knowledge of causes, clinical featuress and diagnosis of common zoonotic diseases among medical practitioners in Tanzania

9.1   Summary

Objectives: To assess the knowledge of medical practitioners of the common zoonoses in Tanzania

Study design: Cross-sectional survey.

Methodology: An open-ended questionnaire was administered to medical practitioners in selected health facilities within urban and rural areas in Tanzania between April and May 2005. Knowledge of medical practitioners of transmission, clinical features and diagnosis of anthrax, rabies, brucellosis, trypanosomiasis, echinococcosis and bovine tuberculosis in humans in urban and rural areas was analysed.

Results: Medical practitioners in rural hospitals had poor knowledge of transmission of sleeping sickness to humans ($p<0.04$) and clinical features of anthrax and rabies in humans ($p< 0.05$ and p-value < 0.01 respectively) compared to the practitioners in urban hospitals. In both areas the practitioners had poor knowledge on how echinococcosis is transmitted to humans, clinical featues of echinococcosis in humans, and diagnosis of bovine tuberculosis in humans.

Conclusion: Knowledge of medical practitioners of zoonotic diseases is likely to contribute to the under-reporting of zoonotic diseases in Tanzania. Refresher courses on zoonoses or more emphasis on zoonotic diseases in teaching curricula of medical practitioners should be implemented in Tanzania to improve the diagnosis of zoonotic diseases. There is also a need for a greater veterinary and medical collaboration in sharing of knowledge important for identification and control of zoonotic diseases.

9.2   Introduction

Zoonotic diseases are diseases that can be transmitted between humans and vertebrate animals (WHO, 1959, Palmer *et al.,* 1998). A total of 61% (n=868) of all human diseases are actually zoonotic and 75% of emerging human pathogens are zoonotic (Taylor *et al.,* 2001). The ability of pathogens to infect a wide range of hosts is a risk factor for disease emergence in humans (Taylor *et al.,* 2001) and animals (Cleaveland *et al.,* 2001). Several emerging human diseases have been cited as transmitted between humans and vertebrate animals (Institute of Medicine, 1992; Morse, 1995; Murphy 1998; Palmer *et al.,* 1998), but its only now that zoonoses have been demonstrated by quantitative analysis as risk factors for disease emergence (Taylor *et al.,* 2001). Both domestic and wild animals have been shown to be important reservoirs of zoonotic diseases (Kilonzo and Komba, 1993; Haydon *et al.,* 2002).

Zoonoses form part of infectious diseases which account for 29 out of the 96 causes of human morbidity and mortality listed by the World Health Organization and World Bank (Murray and Lopez 1996). The connections between animal and human diseases are now generally understood, but individuals as well as societies remain slow to act on this knowledge (Hardy, 2003). This could be due to insufficient systematic continuing education and opportunities to acquire new knowledge on zoonoses for those working in health facilities (Asano *et al.,* 2003). The physician who attends an ill veterinarian or zookeeper will immediately suspect a wide array of other diseases; likewise a pediatrician who attends the child who recently received a puppy for his birthday will not suspect an animal transmitted disease. Medical

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professionals have not been giving due consideration of animals as carriers of diseases that can be transmitted to humans (Goskienski, 1983). This has resulted in poor quality of epidemiological data on different zoonoses and their control measures on animal and human populations in especially sub-Saharan Africa (Holden, 1999; Perry et al., 2002).

Within sub-Saharan Africa, many zoonoses are poorly controlled in both livestock and human populations (WHO, 1994). In Africa, bovine tuberculosis, brucellosis, anthrax, sleeping sickness, and rabies are still widespread (Meslin et al., 1992; Barret, 2006). In a study conducted in northern Tanzania in 2002, nineteen diseases were recorded as zoonoses by household members with rabies, tuberculosis, anthrax and brucellosis the top four zoonotic diseases in pastoral, agro-pastoral and smallholder dairy farming systems (Shirima et al., 2003). Although the majority of households practiced at least one risk activity for transmission of zoonotic diseases there was general lack of knowledge about the diseases. This coupled with poor animal husbandry could be important factors for disease transmission in the areas (Mfinanga et al., 2003, Shirima, et al., 2003).

Although human brucellosis is a modifiable disease in many countries, official figures do not fully reflect the number of people infected each year and the true incidence has been estimated to be between 10 and 25 times higher than what reported figures indicate (WHO, 1997). Cases very often remain unrecognized because of inaccurate diagnosis, and are thus treated as other diseases

as "fever of unknown origin" (WHO, 1997). In Uganda, it was noted that despite the reported increase in the number of individuals infected with *T.b. gambiense species* in the 1990s, WHO estimated that the figures represent only 10-15% of the actual number of infected individuals (Cattand *et al.,* 2001). Apart from poor knowledge of zoonoses, poor referral systems, limited surveillance coverage, difficulty and delay in diagnosis by the health facilities have been contributing to the under-reporting of zoonotic diseases. Patients on the other hand have been seeking alternative services such as those offered by traditional healers and hence delay to present to health facilities or failing to present at all, making data on their diseases not available for epidemiological records (Wandwalo *et al.,* 2000; Cattand *et al.,* 2001; Odiit *et al.,* 2004; Mfinanga *et al.,* 2005).

As targeted education has been demonstrated to be an important factor in improved diagnosis of diseases (Jorge *et al.,* 2005), assessing the level of knowledge of zoonotic diseases of medical practitioners could be an important step in identifying target receptors for public health education in Tanzania. Knowledge of animal reservoirs and their relationship to human disease causation enabled the identification of many zoonotic diseases outbreaks in the world such as Rift Valley fever in Kenya and Somalia (WHO press release, 1998a), Nipah virus in Malaysia and Singapore (Chua *et al.,* 2002), West Nile virus in USA (Lanciotti *et al.,* 1999) and Hendra virus in Australia (Westbury, 2000). No study however, has been conducted in Tanzania to evaluate the knowledge of health personnel responsible for the diagnosis and

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treatment of zoonotic diseases. Here we evaluate the knowledge of medical

practitioners as a factor for the diagnosis of zoonotic diseases in Tanzania.

9.3   Materials and methods

9.3.1   Types of health facilities in Tanzania

All public health facilities in Tanzania are administered by the Ministry of Health,

including privately owned health facilities such as those run by churches. Referral

hospitals manage cases refered from regional, district, ordinary hospitals, health

centres and dispensaries.

Diagnosis and treatment of patients is the responsibility of a range of health personnel

including medical officers, assistant medical officers, and clinical officers (medical

assistants). Medical officers have training to degree level while assistant medical

officers and clinical officers have training to diploma and certificate levels

respectively. For the purpose of this study, all these categories of staff have been

referred to as medical practitioners or practitioners.

9.3.2   The study area

The study was carried out in the districts of Ngorongoro, Karatu and Arusha in

Arusha region, Mbulu, Babati and Simanjiro in Manyara region and Moshi in

Kilimajaro regions in north-eastern Tanzania, and Dodoma urban in Dodoma region

in central Tanzania (Figure 9.1). Health facilities involved included Mount Meru,

Karatu Lutheran and Endulen hospitals in Arusha region and Dareda missionary, Mbulu district, Babati district hospital and Simanjiro health centre in Manyara region. Others included Makole health centre and Dodoma regional hospital in Dodoma region and Mawenzi hospital in Kilimanjaro region (Figure 9.2). All the regions studied have the majority of people practicing animal husbandry (Ministry of Agriculture and Cooperatives, 1995a). Arusha and Manyara regions have subsistence farmers practicing both agro-pastoral and pastoral farming systems whereas farmers in Kilimanjaro and Dodoma regions practice mainly agro-pastoral system.

Figure 9. 1: Map of Tanzania showing study area
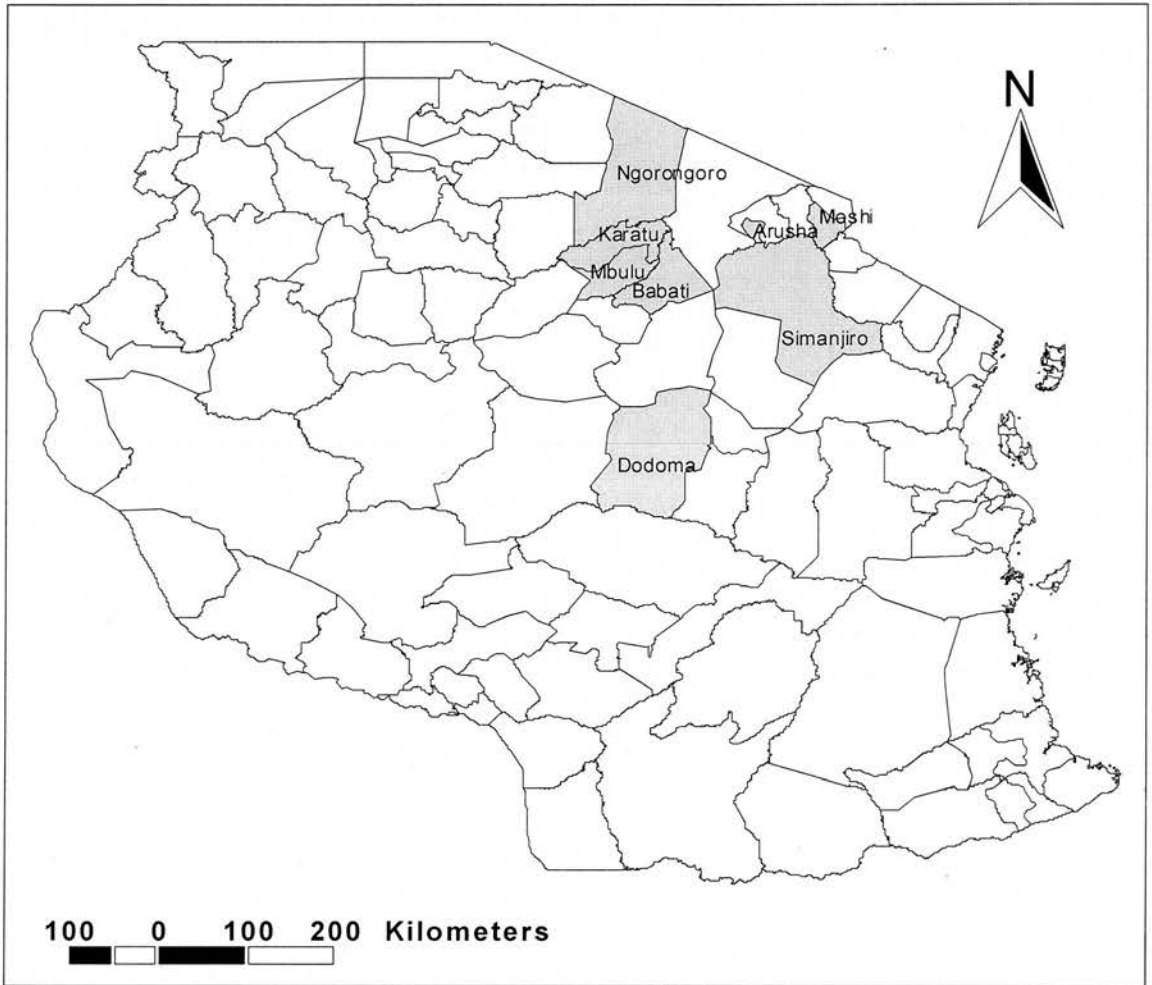
Figure 9. 2: Study area with hospitals involved
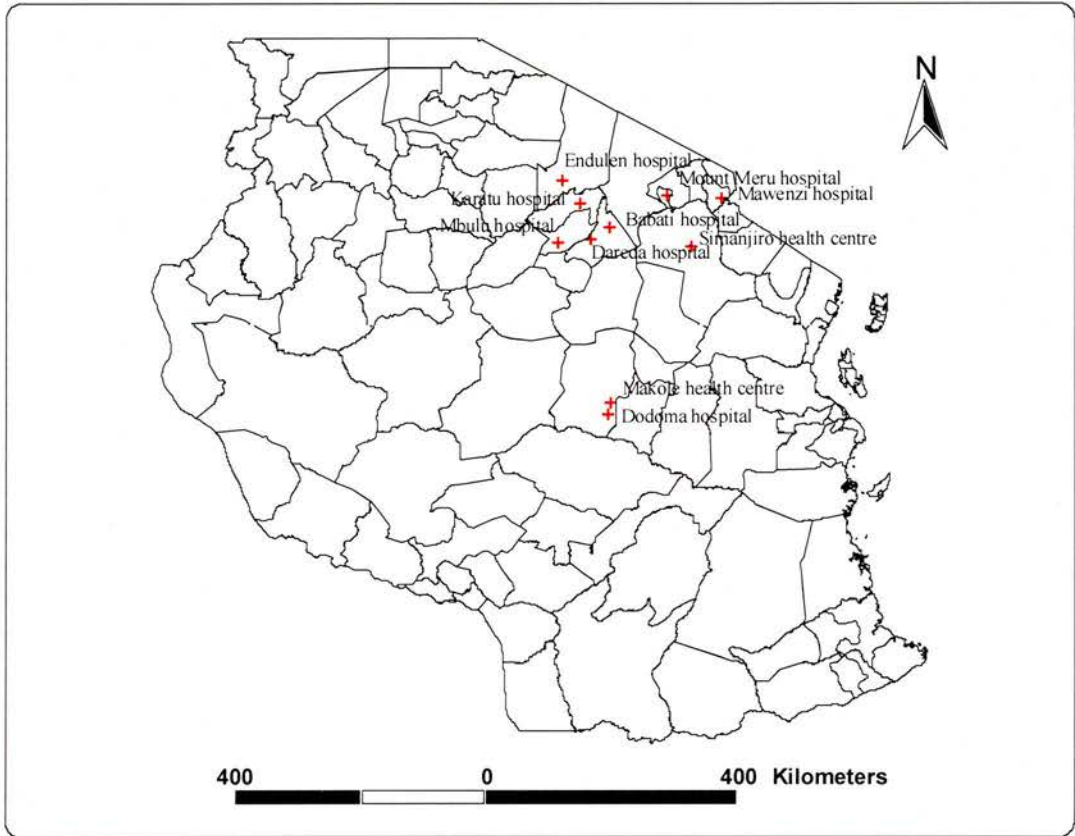
9.3.3   Study design and sampling

The study was designated as a cross-sectional survey and was conducted between April and May 2005. It focuses on areas in Tanzania containing a high proportion of livestock-keepers (Ministry of Agriculture and Cooperatives, 1995b, Tanzania population and housing census, 2002) within the central belt and northern Tanzania. Logistic and time constraints prevented access to regions in the southern highlands. A list of all medical facilities within the regions was compiled and assigned as urban or rural on the basis of Tanzania government regional administrative divisions. Four hospitals were selected at random from urban and six from rural communities within the six regions.

9.3.4   Data collection

An open-ended questionnaire was developed to assess knowledge of the causes, clinical features and diagnosis of anthrax, bovine tuberculosis, trypanosomiasis, rabies, hydatidosis and brucellosis (Appendix V). Field testing of the questionnaire was conducted at Wasso hospital in Ngorongoro district which was not included in the study. The focus of the questionnaire was on medical practitioner's knowledge considered important for the diagnosis of zoonotic diseases. On transmission, the emphasis was on knowledge of animal reservoirs and transmission routes and on clinical features, questions were asked about classical and pathognomonic features of zoonoses in humans and finally data were collected on knowledge of diagnostic protocols for zoonoses.

9.3.5 Data analysis

All the responses were assessed in relation to the information provided by text books of zoonoses (Hubert *et al.*, 1975; Martin and Hugh-Jones, 1995) and were assigned as: "True" if the response was the same or closely similar to the documented, "False" if it was not and "Partial" if the respondent had some correct knowledge of a particular aspect of a zoonotic disease and incorrect on the other aspect. All the practitioners present on the first day of the visit and who agreed to participate with the study were enrolled. Two medical practitioners dropped out of the study citing time constraint. Time to fill in the questionnaire was allocated according to average time recorded during pre-testing (20 minutes). This was done to minimize sharing of knowledge and referring to text books that could have interfered with the analysis.

Medical practitioners were classified into levels of training as medical officers, assistant medical officers and clinical officers and then assigned into urban and rural areas according to the location of health facilities. Frequency data were analysed using likelihood ratio chi-square in Minitab version 14 (Minitab Inc. 2000, Release 14 for Windows, State College, Pennsylvania) to compare knowledge of transmission, clinical features and diagnosis of the zoonotic diseases of the practitioners in the two settings. For each analysis, likelihood ratio chi-square p-value of less than 0.05 was considered to be significant. Fisher's exact test was used where expected results were less than 5.

Analysis was initially conducted in two phases. In the first phase responses from medical officers and assistant medical officers were combined and compared with responces from

clinical officers. In this phase, two analyses were conducted. In the first analysis all the "Partial" responses were included as "True" responses and in the second analysis "Partial" responses were omitted and hence only "True" and "False" responses were included in the analysis. In the second phase, medical practitioners were divided into rural and urban and compared with respect to knowledge of different aspects of zoonoses. As above, analysis was conducted with "Partial" responses as "True" responses and repeated when omitted.

9.4   Results

In total, 4 medical officers, 6 assistant medical officers and 27 clinical officers agreed to

participate (Table 9.1). Based on the location of the hospitals seventeen medical practitioners

were classified as working in rural hospitals and 20 in urban hospitals.

Table 9. 1: Medical practitioners involved in the study and hospitals

Hospital	Farming system	Assistant Medical Officers	Clinical Officers	Medical Officers	Total
Babati	Agropastoral		4		4
Dareda	Agropastoral		2		2
Dodoma	Agropastoral	2	2	2	6
Endulen	Pastoral	1	3		4
Karatu	Agropastoral		2		2
Mawenzi	Agropastoral		2	2	4
Makole	Agropastoral		5		5
Mbulu	Agropastoral	1	2		3
Mount Meru	Agropastoral	2	3		5
Simanjiro	Pastoral		2		2
Total		**6**	**27**	**4**	**37**

When level of training was considered in the analysis, there was no significant difference

between the two groups of medical officers combined with assistant medical officers versus

clinical officers in any aspect of knowledge of zoonotic diseases, showing that level of

training was not a factor in determining the type of response. Analysis was therefore

conducted basing on comparison of responses from medical practitioners in urban and rural

hospitals with "Partial" knowledge responses omitted.

9.4.1 Knowledge of practitioners of transmission of zoonotic diseases

"True" responses with respect to transmission of rabies were recorded in a high proportion of practitioners in rural and urban areas (93.75%, 95% CI=69.77- 99.84, n=19 and 94.74%, 95% CI=73.97-99.86, n=19 respectively) with no significant difference between them (Fisher's exact test, p=1). Significantly more practitioners in urban hospitals appeared to have the correct knowledge of how sleeping sickness is transmitted compared to their rural counterparts (χ^2=4.2, df=1, p<0.05). In both urban and rural health facilities, only a few practitioners were observed to have the right knowledge of the transmission of echinococcosis (44.44%, 95% CI=21.5-69.2, n=18 and 23.53%, 95% CI=6.8-49.8, n=17 respectively), with no significant difference between urban and rural sites (χ^2=1.6, df=1, p>0.05) (Table 9.2).

Table 9. 2: Knowledge of practitioners of transmission of common zoonotic diseases to humans.

Variable	Rural								Urban		Likelihood ratio/ Fisher's exact test p-value
	No. gave correct response	%	No. gave wrong response	%	No. gave correct response	%	No. gave wrong response	%			
Transmission anthrax	9	69.23	4	30.77	15	93.75	1	6.25			0.14*
Transmission brucellosis	12	92.31	1	7.69	10	66.67	5	33.33			0.17*
Transmission rabies	15	93.75	1	6.25	18	94.74	1	5.26			1*
Transmission trypanosomiasis	7	46.67	8	53.33	16	80.00	4	20.00			0.04
Transmission bovine TB	11	84.62	2	15.38	15	88.24	2	11.76			1 *
Transmission echinococcosis	4	23.53	13	76.47	8	44.44	10	55.56			0.19

* Fisher's exact test p value

9.4.2 Knowledge of practitioners of clinical features of zoonotic diseases in humans

There was a significant difference between the knowledge of practitioners in the rural and urban hospitals on clinical features of anthrax and rabies. More practitioners in urban hospitals were found to have the correct knowledge of clinical features of anthrax and rabies compared to the practitioners in the rural hospitals (χ^2=4.6, df=1, p < 0.05 and χ^2=6.991, df = 1, p-value <0.01 respectively). In both urban and rural hospitals a few practitioners were observed to have the right knowledge of clinical features of echinococcosis (33.33%, 95% CI=11.82-61.61, n=15 and 47.06 %, 95% CI=22.98-72.2,

n=17 respectively), with no significant difference between them ($\chi^2 = 0.6$, df=1, p>0.05)

(Table 9.3).

Table 9. 3: Knowledge of practitioners on clinical features of zoonotic diseases in humans

Variable	Rural				Urban				Likelihood ratio/ Fisher's exact test p-value
	No. gave correct response	%	No. gave wrong response	%	No. gave correct response	%	No. gave wrong response	%	
Clinical features anthranx	5	35.71	9	64.29	12	75.00	4	25.00	0.03
Clinical features brucellosis	10	66.67	5	33.33	8	50.00	8	50.00	0.35
Clinical features rabies	7	43.75	9	56.25	17	85.00	3	15.00	0.008
Clinical features trypanosomiasis	11	61.11	7	38.89	11	73.33	4	26.67	0.46
Clinical features bovine TB	7	53.85	6	46.15	3	25.00	9	75.00	0.23*
Clinical features echinococcosis	5	33.33	10	66.67	8	47.06	9	52.94	0.43

* Fisher's exact test p value

9.4.3 Knowledge of practitioners of diagnosis of zoonotic diseases in humans

In both rural and urban hospitals, a few practitioners had the correct knowledge of type of samples and investigations to be conducted to rule out bovine tuberculosis (33.33%, 95% CI=4.3-77.7, n=6 and 47.06%, 95% CI=22.9-72.2, n=17 respectively) and the difference was not statistically significant (χ^2=1.8, d=1, p>0.05). There was no significant difference

between the number of practitioners in rural and urban hospitals with correct knowledge of

diagnosis of other zoonotic diseases (Table 9.4).

Table 9. 4: Knowledge of urban and rural practitioners on diagnosis of zoonotic diseases in Tanzania

Variable	Rural				Urban				Likelihood Ratio/ Fisher's exact test p-value
	No. gave correct response	%	No. gave wrong response	%	No. gave correct response	%	No. gave wrong response	%	
Diagnosis anthrax	7	43.75	9	56.25	10	58.82	7	41.18	0.38
Diagnosis brucellosis	13	86.67	2	13.33	9	56.25	7	43.75	0.11*
Diagnosis rabies	5	33.33	10	66.67	9	60.00	6	40.00	0.14
Diagnosis trypanosomiasis	12	75.00	4	25.00	16	84.21	3	15.79	0.68*
Diagnosis bovine TB	2	33.33	4	66.67	8	47.06	9	52.94	0.66*
Diagnosis echinococcosis	12	75.00	4	25.00	15	78.95	4	21.05	0.09

* Fisher's exact test p value

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9.5   Discussion

The diagnosis and hence reporting of diseases depend to a large extent on the level of understanding of the disease. Accordingly, incomplete understanding of reservoirs of diseases has hampered not only reporting but also the control of many diseases such as rabies in Africa (Cleaveland *et al.,* 1995; Bingham *et al.,* 1999). The knowledge of animal reservoir and transmission modes of zoonotic diseases have helped the identification of many zoonotic disease outbreaks (WHO press release, 1998a, Lanciotti *et al.,* 1999; Westbury, 2000; Chua *et al.,* 2002). But it has been noted that, often health personnel focus more on common or endemic diseases and forget diseases such as those transmitted from animals (Goskienski, 1983). Where malaria is endemic diseases such as brucellosis and anthrax can easily be missed because they have similar clinical features (WHO, 1997).

According to the population and housing census conducted in Tanzania in 2002, about 75% of the Tanzanian population live in rural areas keeping more livestock than their urban counterparts (Ministry of Agriculture and Cooperatives, 1995b; Tanzania population and housing census, 2002). One would therefore expect a higher burden and more awareness of zoonotic disease in rural than in urban areas. In the current study however it was found that practitioners in the rural area had poor knowledge on how sleeping sickness is transmitted to humans ($p < 0.05$) and clinical features of anthrax and rabies ($p < 0.05$ and $p < 0.02$ respectively). In both areas the practitioners had poor knowledge on how echinococcosis is transmitted, how it presents, and how to investigate bovine tuberculosis. Poor knowledge on any one of the aspects of the diseases could result in the disease misdiagnosis. Knowing how the disease manifests without knowing

how it is investigated likewise, knowing how diseases are diagnosed without knowing clinical features can result in misdiagnosis as one wouldn't know which disease to investigate.

Practitioners might be equipped with good history taking skills, but adequate knowledge of how zoonotic diseases are transmitted will provide room for key and focused questions leading to definitive diagnosis and hence treatment of zoonotic disease (Huff and Barry, 2003). It is possible that some zoonotic diseases are missed by the staff entrusted with the duty of identifying them. This might be due to forgetting after years of concentrating on diseases that are thought to be more common or the teaching curricula in institutions concerned don't put due emphasis on zoonotic diseases. Some zoonotic diseases investigated by the present study require advanced diagnostic techniques. Before more weight is put on the acquisition of the sophisticated diagnostic techniques, efforts should be made to equip the concerned staff with adequate knowledge of zoonotic diseases to avoid a waste of resources on equipments that will not be properly utilized.

9.6   Conclusion

The study suggests that lack of knowledge of medical practitioners of zoonotic diseases could be a contributing factor to under-reporting of zoonotic diseases in Tanzania. Refresher courses on zoonoses or more emphasis on zoonotic diseases in the teaching curriculum of medical practitioners should be put in Tanzania as part of sensitization for improved diagnosis of zoonotic diseases. This will not only enable medical practitioners in the proper

diagnosis of zoonoses but will also provide them with up-todate knowledge on prevention of the diseases in the community.

More collaboration between veterinary and medical personnel should be emphasized in Tanzania. This should include sharing and exchange of knowledge on various diseases of mutual interest. Working together between veterinary and medical professionals will enable updating each other on knowledge of zoonoses of public health importance and hence assist in identification and control of zoonotic diseases that are increasingly becoming of importance in Tanzania.

10. General discussion and conclusions

10.1 Introduction

Tanzania is among the sub-Sahara African countries that share the largest of the global burden of diseases (WHO, 2002). However, most of the burden of diseases is carried by the poor underprivileged people in the rural areas whose livelihoods depend on livestock (Perry *et al.*, 2002). It is estimated that livestock contribute to the livelihoods of at least 70% of the world's rural poor (Miranda, 2005). The dependence of rural people on livestock for their livelihoods has made them succumb to many of the zoonoses such as brucellosis making their quality of life deteriorate still further (Perry *et al.*, 2002; Coleman, 2002).

The current study was conducted in livestock keeping communities in Arusha and Manyara regions in northern Tanzania. The households visited practice the traditional animal husbandry which in Tanzania is undertaken by more than 97% of the national herd, and most of these are in the northern part of the country (Ministry of Agriculture and Cooperatives (1995b). Among the ethnic groups studied, the Maasai and Barbaigs practice the pastoralist or nomadic system in which they move far with their livestock in search of pastures, while other ethnic groups such as Iraq, Fyomi and Sonjo practiced agropastoral systems and do not move far with their livesock. Studies conducted in different parts of the world, however, have indicated that pastoralist or nomadic systems favour transmission of zoonotic diseases such as brucellosis, hydatidosis and rabies (Magambo *et al.*, 2006; Okoh, 1981; Schantz, 2006).

This is the first large scale study to be conducted on brucellosis in Tanzania. The study

was unique because it used methodology where the study population was selected at

random and tried to address several key issues that have not been highlighted by any

other studies. Such issues include, the evaluation of diagnostic techniques for brucellosis,

health seeking behaviour and clinical characteristics of brucellosis, risk factors for

transmission of brucellosis to humans, and burden of disease using DALYs and direct

and indirect costs of caring for brucellosis cases. The expectations of the study is to

address these key issues and ultimately formulate the best ways to alleviate the burden of

brucellosis and other zoonoses to particularly the poor people in the rural areas whose

dependence on livestock has made them suffer more from zoonoses.

10.2   Socio-demographic characteristics of brucellosis cases

Gender was not found to be significantly associated with brucellosis. Most studies conducted

elsewhere, indicated males to be at a greater risk of getting brucellosis than females (Thakur and

Thapliyal, 2002; Salari *et al.,* 2003; Beggan *et al.,* 2005). In other studies however, higher

brucellosis seropositivity was found in females than in males (Troy *et al.,* 2005; Hussein *et al.,*

2005). Differences in sexes of the members of sample population and different risk exposures in

different places with regard to sex could have contributed to the different results. In the current

study gender was not a risk factor for brucellosis.

Brucellosis seropositivity was found to be higher in individuals of above 15 years of age than

those of 15 years of age and below. In the study area most of the individuals of above 15 years

were actively involved in different home or farm-based activities. These results suggest that these activities pose a greater risk to them than to other age groups that are less involved with such activities. The age group 16-30 years was found to have a greater proportion of seropositive individuals than any other age group. These results are similar to those obtained by Lulu *et al.*, (1988) in which a greater incidence of brucellosis was observed in the young and the middle-aged, with a low incidence among infants and elderly patients. The age pattern of brucellosis cases contributed significantly to the estimation of burden caused by brucellosis using DALYs because younger age groups carry more YLD than the aged (Murray and Lopez, 1994). As the majority of the individuals of the age group 16-30 years are the ones more involved in production activities, brucellosis therefore is likely to cause enormous socio-economic losses at household and national levels.

10.3   Brucellosis and farming systems

There were proportionally more cases of brucellosis recorded in hospitals located in pastoral areas than in hospitals located in agropastoral areas. Although in the cross-sectional study brucellosis seropositivity was found to be higher in pastoral areas than in agropastoral areas, the difference was not statistically significant. In studies conducted by Schelling *et al.*, (2003) and McDermot and Arimi, (2002) brucellosis seropositivity was found to be higher amongst people practicing pastoral or nomadic systems. Difficulties in the control of animal movement in pastoral or migratory populations have made the control of zoonoses extremely problematic. In pastoral or migratory systems practiced by a significant proportion of agricultural population in Africa, large numbers of animals mix and travel long distances potentially contracting and transmitting zoonoses such as brucellosis. These animals may

then subsequently transmit infection to humans. Interventions to prevent the transmission of zoonoses such as brucellosis to humans should, therefore, consider giving priority to pastoral areas which in Arusha and Manyara regions are inhabited by mainly the Maasai and the Barbaigs.

10.4   Diagnostic difficulties

Hospitals in the study area were found to use many antigens for diagnosis of brucellosis. However, all the antigens used at the hospitals and the RBPT supplied by the study team showed low diagnostic sensitivities. Although many factors could have contributed to the low sensitivities of the tests, the results raised a high index of suspicion on the validity of many tests that are used in the diagnosis of not only brucellosis but also other diseases in Tanzania. It is likely that many diagnostic tests do not meet the standards required for the diagnosis of diseases and hence resulting in underdiagnosis of diseases. Adoption of diagnostic tests with low sensitivities and specificities has great repercussions on the wellbeing of the patients in terms of diagnosis, treatment, the costs involved, their social consequences and the economy of the nation as a whole.

Clinical features of brucellosis were found to be similar to clinical features of other febrile or flu-like conditions such as malaria, typhoid, diseases that affect joints, amoeba and tuberculosis, making it difficult to use clinical features to reach definitive diagnosis of brucellosis. The findings are supported by studies conducted in Kenya and Uganda (Muriuki *et al.,* 1997; Mutanda, 1998; Maichomo *et al.,* 1998), where diagnostic difficulties were also encountered due to the coexistence of such diseases. In the present

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study joint pain, backache, headache, fever and fatigue were the most common clinical features in both brucellosis cases and patients suffering from other febrile or flu-like conditions listed above. Co-existence of such diseases in the study area has also made early diagnosis of brucellosis a difficult exercise. The study calls for a need to establish and harmonise the guidelines for diagnosis of brucellosis in Tanzania. The Ministry of Health and research institutions should be at the forefront of this exercise. More research into the the diagnostic tests which use antigens with high sensitivities and specificities need to be carried out. Meanwhile, while this is sorted out, antigen with the brand name Eurocel A[®] which showed a higher sensitivity than the rest of the tests could be used at the hospitals.

On the other hand zonal laboratories could be established in areas where brucellosis is prevalent, or the existing referral hospitals' laboratories could be equipped with more sensitive tests such as the c-ELISA test. The establishment of such laboratories should go hand in hand with training of laboratory staff to man the equipment and perform quality assurance tests. This would minimize the problems encountered during shipment of samples a long distance for confirmation of diagnosis and also improve the diagnostic capacity for brucellosis and other zoonoses in areas where it is prevalent

10.5   Risk factors for human brucellosis

Brucellosis in humans was found to be associated with assisting aborting livestock, people belonging to the Christian religion and closeness of the nearest neighbour. Contact with products of an aborting animal has been shown by many studies as a risk factor for brucellosis transmission (Young, 1983; Kozukeev *et al.*, 2006; Lim *et al.*,

2005; Bikas *et al.,* 2003; Schelling *et al.,* 2003; Cooper, 1992). In most cases, the

placenta was more associated with brucellosis transmission than other products. In the

present study, there was a significant positive association between brucellosis

seropositivity in humans and in goats at the district level. This indicates that goats

within each district were more associated with brucellosis than goats owned by the

individual household. In the study area there a is tendency for people to assist each other

in different activities. It is a common practice for people within the same district to

assist each other's domestic animals during parturition or handling a new born. In

Mongolia, a study conducted by Zinsstag *et al.,* (2005a) also showed that a significant

proportion of human brucellosis was small-ruminant derived. It is likely that one could

contract brucellosis from an animal that is not his. In the current study the prevalence of

brucellosis in cattle and in sheep also showed positive association with the prevalence of

brucellosis in humans at the village and the district levels but their association was not

statistically significant.

Religious background was found to be a risk factor for brucellosis with people

belonging to the Christian religion more at risk of getting brucellosis than any other

religious group. The sample studied did not allow the comparison between Christians

and other religious groups with respect to risk exposures. However, factors such as

animal husbandry, the number of livestock kept, the location, the interaction between

livestock and humans etc. amongst Christians as opposed to other groups (Muslims or

atheists) need to be studied further. No other study has shown an association between religious belief and risk of diseases.

10.6   The burden of disease caused by brucellosis

Brucellosis is known to cause chronic debilitation to humans if diagnosed late (Corbel, 1997). In the current study, diagnostic difficulties, accessibilty of health facilities, and limited knowledge of practitioners of the zoonotic diseases might have contributed to the late diagnosis of brucellosis cases. Ultimately there was prolonged human suffering from brucellosis. On average cases continued to have brucellosis clinical features for a period of over two years in the current study, out of these, five days as hospital inpatients. During the period of two years, the productive force of the cases was lowered. Each household used a total of US $ 90.65 (92, 826 TShs.) to care for a single case of brucellosis per year and each health provider used a total of US $ 858 (878,592 TShs.) per year to care for cases of brucellosis. Since the presence of infection in man reflects the infection in livestock (Young, 1995a; Karimi *et al.,* 2003; Mishal *et al.,* 1999), humans have to incur the costs of seeking treatment of the animals and bear reproduction and draught power losses as well. All these add further burden to a poor farmer, particularly in rural areas and the health sector that is underfunded.

Brucellosis contributes to poverty and suffering particularly to the people in rural areas who make over 75% of the total population of Tanzania (World Bank, 2002). Based on hospital data 3,637- 8,220 of DALY burden was estimated to be caused by brucellosis and

based on data from the cross-sectional survey 687,861 of DALY was estimated to be caused by brucellosis. Previos studies conducetd in Tanzania showed that *Mycobacteria bovis* caused 9,739 DALY loss, rabies 42,669 DALY burden, *Mycobacteria tuberculosis* (due to human to human transmission) resulted in 730,891 DALY burden in the year 2000 (Coleman *et al.,* 2004). From the data it is evident that the DALY burden caused by brucellosis is not very different from the DALY burden caused by other diseases. However brucellosis is underestimated and neglected.

10.7   Conclusion and recommendations

10.7.1 Community

Health education to the public should be the core of intervention strategies to prevent the transmission of zoonoses such as brucellosis to humans. These should include emphasis on patients to attend hospital early for diagnosis and treatment as late presentation makes diagnosis and treatment difficult. As regards to brucellosis, health education on ways to prevent brucellosis transmission through contact while assisting animal delivery is also required.

The intervention to prevent transmission of brucellosis should give priority to pastoral areas where higher seropositivity of brucellosis was found and should highlight the importance of goats as the main source of *Brucella* infection in Arusha and Manyara regions.

10.7.2 Practitioners

Medical practitioners' knowledge of zoonotic diseases is an important factor in the diagnosis and management of zoonoses. Refresher courses on zoonoses or more emphasis on zoonotic diseases in the teaching curriculum of medical practitioners should occur in Tanzania for improved diagnosis of zoonoses.

It was shown that, as antibodies to *Brucella* can persist for a period of more than 12 months after treatment, practitioners should be made aware of the implications of persistent antibodies on diagnosis and treatment in an area where other febrile conditions of similar clinical features co-exist. Thorough history taking to distinguish between the cured, re-infected, exposure to other febrile conditions and the stage of the disease should be undertaken for proper diagnosis and treatment.

10.7.3 Government

Data recording is an area that needs to be updated and strengthened in Tanzania so that zoonoses such as brucellosis can be notified at district, regional and national levels for better epidemiological data. Currently many zoonoses are not recorded at regional and national levels which is a major omission in data recording systems in Tanzania.

10.8   Future research focus

The current study identified important gaps that require further research. These included studying the efficacy of different brucellosis treatment regimens, studying the *Brucella*

strains common in areas where brucellosis is prevalent, and studying factors associated with Christian religion that might be a risk for human brucellosis.

Estimation of burden caused by diseases is something of utmost importance in health planning of a poor resource country like Tanzania. Estimation of a disease burden enables prioritization of resource allocation and intervention strategies against diseases. It was however noted that, not many disease burdens have been studied in Tanzania using the WHO recommended DALYs. The study challenges the researchers and public health professionals in Tanzania to work on this area based on their disease specialties. Unlike a study conducted in Zimbabwe by Chapman *et al.*, (2006), zoonoses should be given an upper hand and the estimation should include the cases in the community as those presenting to health facilities do not represent the actual number of those diseases.

Future research should also try to explore the benefits of different intervention strategies which might be useful in the control of brucellosis in animal population inTanzania. Such intervention strategies as mass vaccination of animals have proven to be beneficial in some countries (Roth, 2003) if there is a proper allocation of resources and involvement of various sectors dealing with human and animal health.

10.9   Collaborations

The study calls for a stronger collaboration between the veterinary and medical sectors. It was noted that every sector has been taking care of its client, the veterinary sector the animals and the medical the humans. In such cases, zoonoses have been left between the

two sectors with little or no attention at all. Elsewhere such a collaboration has been stressed in various areas of mutual interest (Coulibaly and Yameogo, 2000; Kahn *et al.,* 2006, Zinsstag *et al.,* 2005b; Schelling *et al.,* 2005). These include the clinical area, in which the input from both professions would improve assessments of the risks posed by all species of animals that are in close contact with humans. In public health, human and animal disease surveillance systems would be strengthened in tracking and controlling zoonoses and in research, the dynamics of different disease agents-host interactions can be studied by professionals from both sectors for better prevention and control strategies of zoonoses (Kahn *et al.,* 2006, Fevre *et al.,* 2006).

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12. Appendix

Appendix 1

CASE-CONTROL QUESTIONNAIRE ON BRUCELLOSIS (UNDULANT FEVER) IN HUMANS

1. Background data

1.1 ID no.....................(*Match with tube number for cases, for controls should be allocated*)

1.2 Date of interview (dd/mm/yy)...........................

1.3 Case/control name...

1.4 Sex.............

1.5 Age (years).....................

1.6 Marital status.................

1.7 Tribe of case/control...............

1.8 Religion......................

1.9 Occupation of case/control...........................

1.10 Head of household.........................

1.11 District....................Village.....................

   Sub village......................Ten cell leader.....................

1.12 Interviewee's name...............................

1.13 Sex......................

1.14 Age (year)..........................

1.15 Relationship to the case/control...........................

1.16 GPS coordinates of household.........................S.........................E

1.17 Distance to the nearest neighbour...

1.18 Distance to the nearest health facility/GPS coordinates of health facility and it's name
   Name.......................................Distance......................................
   GPS coordinates ....S........................E

1.19 Distance to the village Centre (CCM office as a reference Point)......................... GPS coordinates...............S.........................E

2.0. Hospital going characteristics, questions 2.1-2.4 (For Controls)

2.1 Have you attended to hospital in the last 5 years? **Yes/No**

2.2 If yes, do you remember which hospital did you go, when and what was troubling you?
....
....
....

2.3 If no, would you go to hospital now if you get prolonged headache, backache, etc? Yes/No/NA

2.4 If no, where would you go and reasons
(NA)..
....

2.5 Are there any family members who got sick but didn't go to hospital? **Yes/No**

2.6 If the answer above is yes, fill the table below **(NA)**

Name	Status (husband/wife/son/ Daughter	Age	What condition was he/she suffering from
1)			
2)			

2.7 Are there any family members who got sick in the same period and went to hospital? **Yes/No**

2.8 If the answer above is yes, fill the table below **(NA)**

Name	Status (husband/wife/son /daughter	Age	What condition was he/she suffering from
1)			
2)			

For cases only

2.9 Where did you go first after getting Sick?..

2.10 If the above facility fails where do you opt for?...............................

3.0 Awareness of Brucellosis

3.1 Have you heard of the disease called Brucellosis in humans? **Yes/No**

   If yes, how it is transmitted/prevented?...

....

4.0 Clinical information

4.1 Has any family member been diagnosed as suffering from Brucellosis /had fever, headache, joint pain, backache? **Yes/No.**

If yes where were they diagnosed?

Name	Status (husband/son/etc.	Age	When 1st get sick Month/year	Has now recovered (Yes/No)
1)				
2)				

Which of the following clinical signs did he/she note:

Coughing ☐   Headache ☐   Backache ☐   Joint aches ☐

Diarrhoes ☐   Fatigue ☐   Anorexia ☐   Fever ☐

Recurrent Fever ☐   Skin disease ☐   Other (describe)........................

5.0 Risk Factors
5.1 Do you keep animals? **Yes/No**...
5.2 If yes, how many of the following:

Animal	No. Adults	No. Calves/kids
1. Goats		
2. Cattle		
3. Sheep		
4. Pigs		
5. Other		

5.3 Were there any abortions in the last one-year? **Yes/No**

5.4 If yes, how many abortions were there? **N/A** Cattle… Goats…Sheep…
Were you involved in disposing the aborted material or assisting the animal?

Yes/No/NA………If yes, how many were you involved in?…………………

Describe how many you were involved in? **N/A** …………………………
………………………………………………………………………………………

5.5 Was there any animal that had retained placenta **Yes/No**?

5.6 If yes, how many retained placenta were there? **N/A**
Cattle………Goats………..Sheep………,

Were you involved in assisting to remove the membranes/disposing them?

Yes/No/NA……………..If yes, how many were you involved in?
Describe……..………………………………………………………………………
………………………………………………………………………………………

5.7. Did you assist animals during parturition? **Yes/No**

5.8 Have you resuscitated a weak born calf/goat/sheep? **Yes/No**, if yes, Describe how

…………………………………………………………………………………………

…………………………………………………………………………………………

5.9 Did you milk cattle? **Yes /No**

If yes, describe……………………………………………………………………

…………………………………………………………………………………………

5.10 How often did you assist in milk cattle? **(NA)**

Daily	Occasionally (describe)	Not applicable

5.11 Did you milk goats? **(Yes/No)**
If yes, describe…………………………………………………………………..

....

5.12 How often did you assist in milking goats? **(NA)**

Daily	Occasionally (describe)	Not applicable

5.13 Did you herd cattle? **Yes/No**
    If yes, how often did you herd cattle? **(NA)**

Daily	Occasionally (describe)	Not applicable

5.14 Did you heard goats/sheep? **Yes/No**
    If yes, how often did you herd goats/sheep**? (NA)**

Daily	Occasionally (describe)	Not applicable

5.15 Were you taking anything when going for grazing? Food:
    Yes/No/NA...................
    Water **(Yes/No)**, Soured milk **(Yes/No)**, other (specify).............................

5.16 If no, how did you manage to stay long without eating/drinking? **(NA)**
    ....
    ....
    ....

5.17 Did you participate in plastering the walls? **Yes/No**
    If yes, how many times/Frequency......................................
    Describe...
    ....

5.18 Did you participate in manure handling? **Yes/No**
    If yes, how many times/Frequency.................................
    Describe...
    ....

5.19 Did you drink cow's milk **Yes/No**......................................

5.20 If yes, could describe the source and frequency in which you consumed cow's milk? **(NA)**

Source	Own cattle	Shop	Neighbour	Relative	Market	Others (specify)
Yes/No						
Freguency i.e.week/day						

Any additional information on milk drinking pattern and seasonality

....
....
....
....

5.21 Did you drink goat's milk **Yes/No?**...................................

5.22 I yes, could you describe the source and frequency in which you consumed goat's milk? **N/A**

Source	Own goat	Shop	Neighbor	Relative	Market	Others (specify
Yes/No						
Frequency i.e.week/day						

Any additional information on milk drinking pattern and seasonality

....
....
....

5.23 How often did you consume? *(Probe in instances where there is plenty of milk or When there is scarcity of firewood if they really boil milk)*

Type of milk	Frequency/ Ranking	Describe
Fresh raw		
Fresh boiled		
Soured raw		
Soured boiled		

5.24 What other food did you eat in which milk was a component?

Food	Yes/No
Ugali	
Makande	
Porridge (uji)	
Tea	
Others (specify)	

5.25 Did you participate in?

Activity	Yes/No	Frequency/Number of times
Slaughtering animal		
Preparation of meat		
Skinning of animals		

5.26 Did you eat Meat? **Yes/No**

5.27 If Yes, in which state did you eat?

State of meat	Rank	Describe organs, how prepared and seasonality consumption
Cooked		
Uncooked		

5.28 Where did you get meat that you ate?

Source	Rank
Shop	
Home slaughter	
Neighbour	
Auction market	
Wildlife	
Others (Specify) e.g.	

5.29 Did you drink blood? **Yes/No**……………………..

5.30 If yes, where did you get blood you drank? **(NA)**

Where blood obtained	Frequency/rank
Bled from live animal	
From slaughtered animal at home	
From neighbour	
From butcher	
From livestock auction market	
From relatives	
Others (Specify)e.g.	

5.31 How was blood prepared before you consumed? **(NA)**

Blood preparation	(V/P/X)
Just take raw	
Raw blood mixed with hot soup	
Raw blood mixed with milk	
Raw blood mixed with duodenal Content, bile and offal chops like river	
Fried/cooked	
Raw blood mixed with ruminal fluid (used as medicine)	
Others (Specify) e.g.	

5.32 Do you remember consuming reproductive organs of animal in the last 1 year? **Yes/No**

5.33 If yes, how were they prepared? **(NA)**

Preparation	V/P/X
Eaten raw	
Boiled	
Mixed with other items and cooked	
Mixed with other items and eaten raw	
Roasted	
Others (Specify)	

5.34 Did you eat aborted foetus/still birth? **Yes/No**

If yes, how many times..

Were you involved in the preparation of this food? **Yes/No/N/A**........................

How many times were you involved in the preparation of this food?.................

5.35 Did you handle any aborted material from wildlife? **Yes/No**..........................

If yes, how many times did you handle materials from wildlife?..

5.36 What wild animals have been coming close to your premises/house

Animal	In the premises	In the grazing grounds	Direct observation
Digidigi			
Nyumbu			
Nyati			
Punda Milia			
Tembo			
Swala Pala			
Swala tomi			
Twiga			
Simba			
Chui			
Fisi			
Bweha			

Nguruwe pori			
Nyani			
Tumbili			
Panya			
Other (mention)			

BRUCELLOSIS QUESTIONNAIRE
For all patients reporting symptoms consistent with brucellosis

Patient ID Number: .... Patient Name: ....
(to match number on serum sample)

Age: .... Sex:.................. Village: ....

Balozi: .... District: ....

A. First Clinical Signs   Date clinical signs first observed: ----/----/------

Which of the following clinical signs did the patient note:

Coughing ☐   Headache ☐   Backache ☐   Joint aches ☐

Diarrhoea ☐   Fatigue ☐   Anorexia ☐   Fever ☐

Recurrent Fever ☐   Skin disease ☐   Other (describe) ....

B. Effect on everyday activities

What is the principal occupation of the patient? ....

What impact does the disease have on everyday activities?

Completely unable to work/go to school ☐   Reduced ability to work/go to ☐
school

No effect ☐   Other (describe) ☐
....

C. First treatment received   Date of first treatment: ----/----/------

Where did the patient first seek treatment? (Tick only one box)

At home ☐   Local healer ☐   Dispensary ☐

Hospital (give name) ....

What treatment was given at the first site of treatment? (i.e. at place ticked above)

☐   Herbal/local treatment (describe) ....

☐ Conventional medicine (describe) ....

....

☐ Other (describe) ....

D. Presentation at hospital   Date of first presentation at hospital: ----/----/----
-

Did the patient suggest himself/herself that he/she may be suffering from brucellosis?
YES/NO

Observations recorded on clinical examination: Temperature   ....^{0}C

Fever ☐   Lympahdenitis ☐ Joint pain ☐ Back pain   ☐   Respiratory signs ☐

Chest pain ☐   Abdominal pain ☐ Neurological signs ☐   Headache ☐

Other (describe) ....

Describe any observations in detail if
necessary..

....

E. Diagnosis and Treatment

1. First differential diagnosis .... Diagnostic test(s) performed:

....Date of test: ----/----/------

Treatment given: .... Date treatment started: ----/----/------

For how long......................

2. Second differential diagnosis .... Diagnostic test performed:
....

.... Date of test: ----/----/------

Treatment given: .... Date treatment started: ----/----/------

For how long......................

3. Second differential diagnosis .... Diagnostic test(s) performed:...............

.... Date of test: ----/----/-----

Treatment given: .... Date treatment started: ----/----/------

For how long......................

4. Third differential diagnosis .... Diagnostic test(s) performed: ....

.... Date of test: ----/----/------

Treatment given: .... Date treatment started: ----/----/------

For how long......................

Name of Clinician: ....

F. FOLLOW-UP (by Brucellosis Project staff)

Date of follow-up: ----/----/------   Was the home of the patient located? YES/NO

Was patient present? YES/NO   If no, who is providing information about patient

....

Response to treatment:

How long did the patient stay in hospital? ....

Has the condition of the patient improved? YES/NO

If Yes:
When did the patient first notice signs of an improvement?
....

Has the patient now fully recovered? YES/NO

For how long did the patient take the medicine?
....

If No:
Has the condition got worse than it was when first reporting to the hospital? YES/NO

Describe current symptoms:

....

....

....

Has the patient sought other treatment since the first hospital appointment? YES/NO

Where? ....

Describe treatment:

....

....

What has been the response to this treatment? ....

....

For all patients:
Were there any adverse effects of the treatment? YES/NO If Yes, Describe ....

....

Is the patient enrolled in the case-control study?   YES/NO

Interviewer: ....

Appendix III
QUESTIONNAIRE FOR CLINICAL SURVEY
Brucellosis project

ID number........................ Name of the patient...

Sex................................. Age..

Tribe.. Religion..

Village.......................................

Ballozi...

District..................................

GPS Coordinates of the household..........................E..............................S

GPS Coordinates of the hospital...........................E...............................S

Name and level of the
facility..

Date of follow-up........................ If patient present Yes/No, if not around who is providing information.............................

Date clinical signs first observed..

Signs and symptoms: Cough ☐ Headache ☐ Backache ☐ Joint pain ☐

Diarrhea ☐ Fatigue ☐ Anorexia ☐ Fever ☐ Recurrent fever ☐

Skin disease ☐ Other ☐...

Occupation of the case/control..

Was the patient able to perform the following activities?

Activity	Completely unable	Reduced ability	Able
Maintain the farm			
Looking after livestock			
Going to market			
Prepare family meals			
Collection and chopping fire woods			
Plastering walls			
Collecting water			
Looking after children			
Other			

Date first treated……………………..,. Where treated….……………………..

Time spent in going to hospital……..…..…………., time spent at the hospital………
…………………………………………………………………………………………

Give costs for each of the following items
Registration
Investigation
Drugs
Fair
Other costs

Name of person who went with you to hospital………………… Sex……..Age………
Who is he/she to you…………………… cost incurred for his/her transport…………..

What was the diagnosis……………..

What was the treatment given:

Local herbs……………………………………………………
Conventional medicines…………………………………………
Other…………………………………………………………

Date of first presentation to hospital……………………

If suggested to Clinician to be investigated for brucellosis, Yes/No, if yes why?………
…………………………………………………………………………………………

Time spent in going to hospital……..…..…………., time spent at the hospital………
…………………………………………………………………………………………

Give costs for each of the following items

Registration
Investigation
Drugs
Fair
Other costs

Name of person who went with you to hospital..................... Sex........Age.........
Who is he/she to you....................... cost incurred for his transport.................

Day you started treatment...................................., what treatment was given......
....

If has improved, Yes/No, if improved when started to experience
improvement.............

If fully recovered Yes/No

For how long did you take the medication?....................

If not fully recovered what are the symptoms..
....
....

If patient sought further treatment, Yes/No, If yes where.....................................

When treated..........................., What was the diagnosis...........................

What was the treatment given...
What was the response...

Were there any adverse effects of any medication given above?
Yes/No...................... If yes, explain the effects...
....

If any at home ever suffered from brucellosis Yes/No, if yes;

Name	Status	Age	Sex	Symptoms

Socio economic status of the case/control

Number of houses…………….. number of hurts it has…………………………………….

Number of wives………………… Number of

children…………………………………….

Look at the house and observe

Roofing………………………………………………………………………………………….

Walls………………………………………………………………………………………….

If has: radio………….. number of speakers it as………………..Bicycle………………..

Tractors………………,Cars…………………….,

Item	Percentage contributing to income	What used for
Salary		
Crop selling		
Sheep selling		
Cattle selling		
Goat selling		
Other		

Amount used for food, clothing and medication for animals and humans in a month	
Amount used for Education for children in a year	

How much of the following were harvested last season?

Maize...............................
Beans...............................
Millet...............................
Other...............................

Where do you get treatment for your animals

Community based animal health worker	
Local livestock field officer	
District veterinary officer	
Self treatment	
Traditional/herbs	
No treatment	

Level of education reached

No formal education	Primary	Secondary	College	Other

If experienced any type of stigma...
....
....
....
....
....
....

APPENDIX IV

LABORATORY FORM FOR BRUCELLOSIS

Sample No.	Name of patient	Age	Sex	Village	Balozi	Hospital Registration number	Results Rose Bengal Test	Results Chronolab/other hospital Test	Date of test

Appendix V

Questionnaire on zoonoses – Doctors and clinicians

ID No...

Hospital.....................................

District.....................................

Status-MO/AMO/MA/RMA............

1. Anthrax, brucellosis, rabies, trypanosomiasis, echinococcus, cholera and bovine tuberculosis are known to be common in your area, we would like to know your comments on the following:

a) Briefly describe the way the following zoonoses are transmitted, stating the animal reservoir and how transmitted:

    i) Anthrax..

....

....

    ii) Brucellosis...................................... ....

....

....

    iii) Rabies ....

....

....

iv) Trypanosomiasis...

....

....

....

....

v) Bovine tuberculosis...

....

....

vi) Echinococcus ....

....

....

vii) Cholera...

....

....

2) What do you think are the clinical presentations of the diseases (signs and symptoms)

    i) Anthrax...

....

....

    ii) Brucellosis...

....

....

iii) Rabies ....

....

....

iv) Trypanosomiasis..

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....

....

v) Bovine tuberculosis..

....

....

vi) Echinococcus ....

....

....

vii) Cholera..

....

....

3) What investigations would you do to diagnose the following above zoonotic diseases

i) Anthrax...

....

....

ii) Brucellosis...

....

....

iii) Rabies ....

....

....

iv) Trypanosomiasis...

....

....

v) Bovine tuberculosis...

....

....

vi) Echinococcus ....

....

....

vii) Cholera...

....

....

DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS OF BRUCELLOSIS IN ARUSHA AND MANYARA REGIONS, TANZANIA

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Abstract: A review of hospital records and interview with district medical officers and health staff in Karatu, Ngorongoro, Babati, Hanang and Mbulu districts in Arusha and Manyara regions was conducted between July and September 2002. A data analysis showed that hospitals use different antigens and techniques to test for brucellosis. Some use titration method and others serum/rapid agglutination tests. *Chronolab* with *Brucella abortus* antigen was the most commonly used technique. Other antigens included *Merox* with *B. melitensis* and *Biosystem* with *B. melitensis* and *B. abortus* antigens. Most of the hospitals that employ titration method used titre of 1:80 and above to diagnose brucellosis. There was a wide disparity in treatment regimens and costs between different health facilities. Diagnosis and treatment of brucellosis need to be addressed urgently. To do this, it is important that different treatment regimens and costs for brucellosis are evaluated in order to determine the most efficacious and cost effective regimen. It is also important that the government provide adequate drugs for the treatment of the diseases to avoid irrational use of antibiotics that might predispose to emergence of drug resistant strains. Evaluation of different diagnostic tests should be done so as to establish the most sensitive and with reasonable specificity. In this paper, the diagnostic, therapeutic and cost implications of brucellosis in two regions of northern Tanzania have been discussed.

BRUCELLOSIS IN ARUSHA AND MANYARA REGIONS, TANZANIA: A CHALLENGE TO PUBLIC HEALTH

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Summary

Setting: Karatu, Ngorongoro, Babati, Mbulu and Hanang districts in Arusha and Manyara regions, Tanzania.

Methodology: A retrospective study was conducted in Karatu, Ngorongoro, Babati, Hanang and Mbulu districts in Arusha and Manyara regions between July and September 2002. Review of Hospital records and interview with district medical officers, hospital Incharge and clinicians were conducted.

Results: Out 170,345 patients who attended hospitals in Babati, Dareda, Karatu, Hydom, Katesh, Wasso, Endulen and Mbulu hospitals in the year 2001, 619(0.36%) were diagnosed as having brucellosis. It was found out that women suffer from brucellosis more than males. Out of 619 cases of brucellosis reported in the year 2001, 432 (69.8%) were females and 187(30.2 %) were males. Most of the patients were of the age between 16-35 (46.3%) i.e. after school age compared to the middle aged 36-50 (30.4%). Few cases were found in the pre school age (<7 years), school age (7-15) and old age (>50 years)

Conclusion There is a need to carry out a study that will establish the burden caused by brucellosis in the area. This should also include investigating the relationship between infection in animals and that in humans. There is also a need to investigate the burden of the disease to the community as well and not only to those who attend hospitals . Factors that favor acquisition of brucellosis to animals and subsequent transmission to humans must be explored and pointed out clearly so that the communities can be made aware of and hence minimize the chances for transmission of brucellosis

Introduction

Brucellosis is an acute or chronically contagious disease of animals and humans, caused by species of the genus *Brucella* (*Brucella abortus, B suis, B. melitensis, B. ovis, B. neotomae*). The disease has different names; mediterranean fever, malta fever and undulant fever have been assigned to humans *(Corbel, 1989)*.

Although brucellosis can be found worldwide, it is more common in countries that do not have good standardized and effective public health and domestic animal health progs. Areas currently listed as high risk are the Mediterranean Basin (Portugal, Spain, Southern

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France, Italy, Greece, Turkey, North Africa), South and Central America, Eastern Europe, Asia, Africa, the Caribbean, and the Middle East. Un pasteurized cheeses, sometimes called "village cheeses," from these areas may represent a particular risk for tourists (World bank, 1985).

The incidence of brucellosis caused by *B. melitensis* in sheep, goats and humans is still a very significant problem in Macedonia and Greece. In Greece, cattle are also affected either by *B. melitensis* or *B. abortus*. The disease is an endemic problem in some regions of Yugoslavia and includes *B. suis* biovar 2 in pigs and in Croatia, *B.melitensis* in sheep, goats and humans is found occasionally. No problem appears to exist with brucellosis in Bulgaria. Financial well-supported brucellosis control progs of the European Union that will include all countries, regardless of the magnitude of brucellosis incidence, are needed for eradication and control of brucellosis (Taleski V, 2002).

Brucellosis has been reported in a number of African countries with a varying range of prevalence. The reported prevalence in various countries include 3% in Malawi (Benard,, 1993); 2.27% in Sudan (Mahmoud, 1991); 4.2% in Ethiopia (Tekelye – Bekele 1989); 5.45%-17.5% in Kenya (Waghela, 1977, Ndarathi and Waghela, 1991); 9.5% in Somali (Wernery *et al.*, 1979); 7%-50% in Nigeria (Eze, 1977); 37..9%-61.8% in Egypt (Refai, 1990).

In Togo, studies done in 2000 indicated that brucellosis is among the major zoonoses. A national eradication proge was instigated to reduce the transmission of rabies. Good relations exist between

veterinary and health personnel in the field but this level of interaction is absent at district and national level. This has resulted in information not being transferred between the two disciplines and the lack of a national strategy for the eradication of zoonoses in Togo. This represents the condition in many African countries (Domingo, 2000).

In Tanzania, Evans (1935) first reported brucellosis in humans after reviewing the status of the disease in the then Tanganyika. He stated that cultures obtained from humans had shown that *B. abortus and B. melitensis* occurred in the country. Further reports of human Brucellosis in the country were obtained from monthly reports of the medical department of the Lake region and west region for 1959, 1960 and 1961 and from two patients at Kihesa village in Iringa region in 1962 (Anon, 1963). However, because clinical symptoms of brucellosis in humans are similar to malaria and typhoid (Mutanda, 1998, Muruiki *et al.,* 1997), it is possible that some cases of brucellosis are recorded as malaria or typhoid. These difficulties have contributed to the general lack of information on the disease in Africa (Anon, 1963).

In cattle, brucellosis was first diagnosed in 1928 from samples taken from aborted cattle at Engare Nanyuki (Kitalyi, 1984). The existence of bovine brucellosis in Tanzania arose interest of researchers to study its spread in the country (Anon, 1927). Several surveys have been conducted in different parts of the country (Table 3), with prevalence varying from 5% to 20%. However, these studies were conducted in more intensive farming systems or in response to abortion problems and may therefore not be representative of the majority of the indigenous cattle population, kept under traditional husbandy systems.

A decline in the cattle growth rate from 3.4% in 1974 to 0.6% in 1984 was attributed to among other factors by brucellosis (Anon, 1987), but the evidence is weak, however, brucellosis is thought to remain endemic and widespread throughout Tanzania (Corbel, 1989).

The following is a table (Table 1) showing surveys of brucellosis in animals conducted in Tanzania, all these used serum agglutination test. The findings show that brucellosis has been in Tanzania for a long time at varying degrees.

Table 1. Surveys of Bovine brucellosis conducted in Tanzania

Year of survey	Author	Zone	Prevalence
1962	Mahlau, Hammond	Lake	13.5%
1967	Mahlau	Southern Highlands	13.2%
1969	Hoffman, El Sawah	Western	14.2%
1971	Mahlau	Southern Highlands	20.5%
1973	Staak,Protz	Northern	7.6%- Masai 3.3%- Mbulu
1984	Kitalyi	Central	5.1%
1986	Msanga *et al.,*	Lake	10.6%

The concentration of population in urban and peri-urban areas especially in the last ten years has substantially increased the demand for milk, milk products and other animal products in such areas. This together with the deterioration of veterinary services in the country is creating social, environmental and public health problems within the community. Because the demand is higher than production, some milk vendors and sellers of other animal products are forced to sell un-pasteurized milk and other animal products, which are not or not properly processed (*Corbel, 1989*).

Brucellosis, caused by a variety of *Brucella* species, is a disease of major socioeconomic importance in animals worldwide, especially so in developing countries where disease control proges are either non-existent or inadequate. The disease also occurs in the wildlife, thus posing a danger of transmission between domestic animals and wild animals in interphase areas (Anon, 1927).

Devastation caused by Brucellosis

The disease is associated with abortion storms in newly infected herds; high degree of retained placenta and hence endometritis which often result into reduced milk production and infertility. Costs of treatment, loss of calf crop size and hence replacement of stock and concurrent human infection are other sequel of animal infection (*Corbel, 1989*).

Humans succumb to prolonged periods of general body malaise, headache, backache, fever and chills. Due to long illness, a lot of time is spent in seeking medical help in hospitals and in some areas local/traditional healers instead of participating in economic activities. This coupled with large amount of funds used in treatments, to a large extent worsen the economies of the people.

Arusha and Manyara regions

Arusha and Manyara regions are situated in the northeastern part of Tanzania. Before 2002 they were one region called Arusha. Babati, Mbulu and Hanang districts are in Manyara region, Karatu and Ngorongoro are in Arusha region. The major tribes in Arusha and Manyara regions include Maasai, Mbulus (Irakis), Barbaigs and Sonjos. The Maasai and Barbaigs are primarily livestock keepers, these normally move from one part of Tanzania to another with their cattle in search of pastures (pastoralists). The rest of the tribes are essentially livestock keepers and also grow crops for their food (agro pastorals).

Methodology

A retrospective study was conducted in Karatu, Ngorongoro, Babati, Hanang and Mbulu districts in Arusha and Manyara regions between July and September 2002. Review of Hospital records/data and interview with district medical officers, hospital Incharge and clinicians were conducted. Hospitals visited included Karatu Missionary hospital that serves the community in Karatu district and some parts of Ngorongoro district, Mbulu district hospital in Mbulu, Katesh district hospital in Hanang, Hydom Lutheran hospital that serves Mbulu district and neighboring districts of Arusha, Manyara and Singida regions. Also visited were Dareda missionary hospital, Babati district hospitals in Babati and lastly Wasso and Endulen missionary hospitals in Ngorongoro district. Babati, Katesh and Mbulu hospitals are government owned district hospitals whereas Hydom, Karatu, Wasso, Endulen and Dareda hospitals are missionary hospitals.

Any person who presented to hospital with one or more of non specific symptoms such as fever, headache, joint pain, backache, malaise etc. and who tested positive with serum agglutination test was treated as a brucellosis case. Laboratory diagnosis was based on titres of agglutinins of 1/80 and above in all the hospitals that use titration method.

Results

Brucellosis was found in 619 (0.36%) patients out 170,345 who attended to the hospitals in selected districts in Arusha and Manyara regions in the year 2001. Most of the patients came from Endulen hospital (182 patients) and Dareda hospital (165 patients) (Table 2).

Table 2. Brucellosis patients in the year 2001

Hospita	No. of cases	Males	Females	Total attendance	Proport %
Karatu	100	25	75	26,963	0.4
Mbulu	46	17	29	14,944	0.3
Hydom	51	13	38	88,265	0.05
Katesh	-	-	-	-	-
Babati	40	8	32	8,985	0.4
Dareda	165	46	119	9,297	1.77
Wasso	35	8	27	11,968	0.3
Endulei	182	70	112	9923	1.8
Overall	619	187(30%)	432(70%)	170,345	0.36

Of the brucellosis patients, 415 (67%) were females compared to 204 (37%) males (χ^2 =22.17, df=4, P<0.01). Of these, 34 (5.4%) were 15 years of age and below, i.e. school age, 223 (36%) were 16-30 years of age, 138 (22.3%) were 31-45 years of age, 179 (29%) of age 46-60 and 45 (7.3%) more than 60 years of age (Table 3).

Table 3. Age and sex distribution of brucellosis cases in the year 2001

Age group (years)	Number of patients			Percentage (%)
	Males	Female	Total	
=< 15	15	19	34	5.4
16 – 30	66	163	223	36.0
31 – 45	60	72	138	22.3
46 – 60	43	136	179	29.0
>60	20	25	45	7.3
Total	204	415	619	100.0

It was observed that most of the cases were seen in the beginning of the year and towards the end of the year, this was more marked in the pastoral areas e.g. data from Endulen hospital. Comparing the two seasons i.e. the first and last quarters versus the middle two quarters of the year there was a significant difference in the number of cases (χ^2= 58, df=6, p<0.01). Cases increase towards the end of the year and remain high in the beginning of the subsequent year when there is rain only to drop in the middle of the year when most of the time it is dry (Table 4).

Table 4. Distribution of brucellosis cases in the year 2001

Hospital	Jan - March	April - June	July - Sept.	Oct. – Dec	Total
Karatu	29	20	23	28	100
Mbulu	7	6	18	15	46
Hydom	9	13	10	19	51
Katesh	-	-	-	-	-
Babati	8	12	9	11	40
Dareda	42	53	30	40	165
Endulen	56	23	39	64	182
Wasso	8	4	9	14	35
Total	159	131	138	191	619

Discussion

Out of 170,345 people who attended health facilities in the study area in 2001, 619 (0.36%) were found to have brucelosis. The proportion of people who attended to the hospitals in the year 2001 is a small proportion of the total population of the two regions that is exposed to getting brucellosis. Since brucellosis runs a chronic course then we expect the seroprevalence of brucelosis in the study area to be much more than what we have obtained. Some patients can remain sick at home without seeking medical help until condition allows them to go to hospital e.g. after they have sold their crops or they don't have any other commitment e.g. cultivation. The majority of cases were reported in the first and last quarter of the year at

this period it is rainy, and animals reproduce, hence there is plenty of milking and drinking of milk, which can be important activities that favor transmission of brucelosis from animal to animal and subsequent transmission to humans.

In most of African cultures women engage more with taking care of animals, milking and most importantly assist animals during parturition, so they are more exposed to getting brucellosis in our setup than males. Most of the patients were of the age between 16-30 years, few people in the Pre School, school and old age were found to be suffering from brucelosis (table 3, fig 1). At the age of 16-30 years most of the people in these areas have finished primary education, are married and actively involved in different home activities.

Relapse cases are not common though in some areas patients attend to hospital soon after completion of medication to be tested for *Brucella*. These may go from one facility to another (hospital shopping) where of course they will test positive if they were initially positive because antibodies will still be there. In some cases where they don't have facilities for diagnosis e.g. antigen or due to reasons not clearly known clinicians or doctors don't routinely request a test for *Brucella*, in this case patients succumb to prolonged periods of illness. It was observed thatSince brucellosis presents with non-specific symptoms that resemble malaria and typhoid, most of the patients have to be investigated for the diseases before they are diagnosed as brucellosis cases. From interview with medical personnel, patients present to hospital late e.g. with symptoms of more than one month, patients start treatment for malaria or typhoid but symptoms persist forcing them to go to hospital for further investigation only to be found to have circulating antibodies against brucellosis.

Conclusion

There is a need to carry out a study that will establish to exactly what extent brucellosis is a problem in Arusha and Manyara regions and investigate the relationship between infection in animals and in humans. Brucellosis runs a chronic course, so there might be a big proportion of people in communities that have brucellosis infection but don't have symptoms that force them to go to hospital. There is a need to investigate the prevalence of infection not only to population that presents to hospitals but also in the community. Mfinanga *et al., 2004* found out that the people in Arusha and Manyara regions have a habit of consuming raw animal products that predispose them to getting tuberculosis. Burcellosis can be transmitted in the same way. Factors that favor acquisition of brucelosis to animals and subsequent transmission to humans must be investigated because we need to educate the community at risk on different ways in which brucelosis can be acquired by both animals and human beings. This will reduce the burden of brucellosis in the area.

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