ESTIMATION OF THE PREVALENCE OF BRUCELLOSIS IN HUMANS AND LIVESTOCK IN NORTHERN TURKANA DISTRICT, KENYA> '

DA VID WAN YON YI NANYENDE (B VM)

UNIVERSITY OF NAIROBI

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University. /Ov 4/

Signed:  - Date: ^ J ^

DAVID WANYONYI NANYENDE

This thesis has been submitted for examination with our permission as the Supervisors.

Signed: Date: U * ^ ^

PROFESSOR S. M. ARIMI (B.V.M, MSc., PhD.)

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

Signed: (t ^ ^) Date: O

DR. P. M. KITALA (B.V.M, MSc., PhD.)

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

Signed: ^ Dat

DR. G. MUCHEMI (R.V.M, MSc., PhD.)

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

DEDICATION

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ABSTRACT

Brucellosis is an important zoonosis especially among the pastoralists like the Turkana who live in close contact with their livestock. A cross-sectional study was performed to determine the sero-prevalence of brucellosis in cattle, goats and humans in the northern part of Turkana District, Kenya and also to identify risk factors for the infection. Serum samples were collected over a period of four months starting October, 2006 to February, 2007. The study area was stratified into three regions reflecting how the area is served by the three major livestock routes. The samples were then collected from the different livestock camps using systematic sampling. The total samples collected were as follows: 200 from cattle, 400 from goats and 174 from humans. All the serum samples were screened using the Rose Bengal Plate Test (RBPT) and thereafter subjected again to Competitive ELISA (cELISA) Test. In addition, using questionnaires, information regarding risk factors for brucellosis in both livestock and human was collected. The risk factors assessed with regard to brucellosis in livestock were: management (grazing and watering) system, introduction of new stock, level of awareness regarding brucellosis and frequency of contact with extension staff. Risk factors assessed with regard to the infection in humans were:- close association with livestock, consumption of raw livestock products such as milk and blood and level of awareness about brucellosis.

In this study, an overall seroprevalence of 17% was observed in humans, 13% in goats and 11% in cattle based on Competitive ELISA test. The Rose Bengal Plate test gave the seroprevalence of 1.7% in humans, 2% in goats and 3.5% in cattle.

The level of agreement for the two tests using the kappa statistic was determined and it showed that there was a moderate agreement in cattle (Kappa = 0.45), and slight agreement in goats (Kappa = 0.24) and humans (Kappa = 0.16)

Using Univariate logistic regression analysis, the major risk factor for seropositivity in livestock was identified as communal grazing of the animals (PO.OOl for cattle and P=0.003 for caprines). In humans, brucellosis was high among the pastoralist group compared to the non-pastoralist population (P=0.007). Consumption of raw blood was also significant (P=0.025). The level of significance was worked out at 95% confidence interval.

The study reveals that brucellosis is widely distributed in northern Turkana in cattle, goats and humans and therefore constitutes an important economic and public health challenge. The study results provide baseline data for future studies of brucellosis infection in Turkana District and a starting point for initiating control measures in both livestock and humans.

1. INTRODUCTION

1.1 Background

Brucellosis is an infectious bacterial disease affecting domestic animals, humans and wild animals. It is of major economic importance and public health significance worldwide.

Brucellosis is widely distributed in Africa with the highest incidence in areas where extensive livestock husbandry is practiced and animal populations are high (Chukwu, 1985; McDermott and Arimi, 2002). It is a zoonosis transmitted directly or indirectly from infected animals to man with consequent debilitation and prolonged incapacitation. The disease has been reported in humans in Turkana District (MOM Annual Reports, 2004 and 2005). From the records at the District Veterinary Office in Lodwar, cases of brucellosis have not been reported in livestock but this is likely because tests have not been carried out to ascertain the cause of abortions and retained placenta (DVO reports, 2001,2002, 2003, and 2004).

Information regarding the prevalence of brucellosis in the country is scanty and disjointed. Diagnostic tests in livestock are carried out at the regional veterinary laboratories. In 2001, there were 34 cases and in 2002, 95 cases in cattle (Director of Veterinary Services Annual Reports; 2001, 2002). There was no report of the disease in other livestock species.

In this study, an estimation of the prevalence of brucellosis was made in humans, goats and cattle in northern Turkana District. Turkana District is inhabited by the Turkana

community who are nomadic pastoralists. Like other pastoralists, livestock play an important and central role in their daily and ceremonial life. They depend on livestock for meat, milk and blood (Barret, 1998). In addition, livestock provide the principal currency for social and commercial transactions (McDermott *et al.,* 1999).

1.2 Justification for the study

The clinical features and presentation of the disease in humans overlap with many other infectious and non-infectious diseases which present 'flu-like' syndromes. There was therefore a need to establish the prevalence of the disease in man.

Lack of pathognomonic signs in livestock presents a challenge in the diagnosis of brucellosis in livestock despite the high risk of the disease in Turkana District. It is possible that some of the cases of abortion reported in the district are caused by brucellosis. This study therefore endeavoured to establish the status of the disease in livestock.

Livestock movement is a major risk factor for brucellosis in livestock. The spread of the disease from one herd to another and from one area to another is almost always due to the movement of an infected animal from an infected herd or area and is therefore a major cause of brucellosis control breakdown. The prevalence is linked to the practice of animal movement to dry grazing areas and mountain pastures where there is commingling of livestock from a variety of sources on the same pasture. Uncontrolled livestock movement is common among nomadic pastoralists such as the Turkana.

Brucellosis is of great economic importance and the losses arising from the disease are enormous for the pastoralists. The economic losses associated with brucellosis include the following:-

a. Decreased milk production by aborting livestock.
b. The sequel of infertility increases the period between lactation and there is prolonged inter-calving period.
c. There is loss of calves, kids and lambs due to storm abortion, stillbirths, weak neonates and deaths. This results in stagnation of livestock population and interference with breeding programs.
d. The resultant infertility leads to heavy culling of valuable livestock.
e. Deaths of cows, ewes and doe as a result of acute metritis following retained placenta (Chukwu, 1987).

Abattoir construction which is underway at Lokichoggio gives the pastoralists an outlet for their livestock. Abattoir workers and those employed in the meat processing industry are at risk of contracting the disease. It is therefore necessary to establish the level of risk to which the abattoir workers will be exposed so that they, together with the consumers, can take the necessary precautions.

The disease if controlled will improve the pastoral economy which relies heavily on livestock as international trade opportunities will be opened up. Currently, any livestock exported must be free from brucellosis. In addition, the disease in humans has an effect on household economy. The disease presents a non-specific clinical picture which results

in a diagnostic problem. This leads to inappropriate treatment, thereby prolonging medical expenses for combating this debilitating disease. A lot of family income will therefore be spent on medical bills for which relapses are very common.

The study was undertaken in Turkana District because it is a typical pastoral population. People live in close association with their livestock and depend on them for food, clothing, ceremonies and commercial activities. Their lifestyle in turn predisposes them to a high risk of infection with brucellosis. Establishment of the prevalence of the disease in the region will therefore form the first step of instituting the necessary measures in preventing and or controlling the disease in livestock and humans. Swift *et al.* (1990) identified brucellosis as being present at high levels in Turkana District.

1.3 Problem statement

Records at the health facilities in Turkana District indicate that humans cases of brucellosis are very common. Records at the District veterinary Office however, do not show any indication of reported cases in livestock although reports of retained placenta, abortion and infertility are common. The study attempts to ascertain that the infection is indeed present in humans and therefore seeks to establish the likely source of infection.

The Turkana community like many other pastoralists live in close association with their livestock and engage in practices which enhance risks to brucellosis infection. Such practices include consumption of raw or poorly cooked livestock products and sharing water sources and even housing with their livestock. This therefore provides the

justification to establish the level of brucellosis in both humans, cattle and goats in order to create a link between livestock and human infection.

1.4 The study objectives

The overall objective of this study was to estimate the prevalence of brucellosis in cattle, goats, and humans in northern Turkana District and suggest possible control measures. The specific objectives were:-

i. To estimate the prevalence of brucellosis in cattle, goats and humans in northern Turkana District.
ii. To determine the risk factors associated with brucellosis in both livestock and humans in northern Turkana District.
iii. To generate maps indicating the distribution of brucellosis in northern Turkana District using Geographic Information System.

2. LITERATURE REVIEW

2.1 Epidemiology of Brucellosis

Brucellosis is a worldwide disease affecting man, domestic animals and wildlife (Chukwu, 1985; Baldi *et al.*, 1994; Hailing *et al.*, 2005). *Brucella spp* infections have been documented worldwide in a variety of terrestrial wildlife species and marine mammals. *Br. abortus* and *Br. suis* have been isolated from bison, elk, feral pigs, wild boar, hares, foxes, African buffalo, eland, waterbuck and may serve as carriers for other domestic animals and humans (Palling *et al.,* 1988; Davies, 1990; Godfroid, 2002). Brucellosis is considered the commonest zoonotic infection in the world (Pappas *et al.,* **2006).**

Brucellosis is widely reported in Africa in all the livestock species and man (Chukwu, 1985; McDermott and Arimi, 2002) and is considered to be endemic (Kubuafor *et al.,* 2000). The disease has been reported in Chad in humans, camels and cattle (Schelling *et al.,* 2003), Togo (Domingo, 2000), Burkina Faso (Coulibaly and Yameogo, 2000), Nigeria (Ocholi *et al.,* 1996), Eritrea (Omer *et al.,* 2000), Ghana (Kubuafor *et al.,* 2000), Zambia (Ghirotti *et al.,* 1991; Ahmadu *et al.,* 1999; Muma *et al.,* 2006), Malawi (Bedard *et al.,* 1993), Ethiopia (Alemayehu, 1981; Seboxa, 1982) Sudan (McDermott *et al.,* 1987) Zimbabwe (Mohan *et al.,* 1996), South Africa (Reichel *et al.,* 1996) Uganda (Mutanda, 1998; Kabagambe *et al.,* 2001) Somalia (Ostenello *et al.,* 1999), Cameroon (Shey-Njila *et al.,* 2005) Tanzania (Weinhaupl *et al.,* 2000; Kunda, 2004), among other countries in Africa.

In Kenya, the disease was first reported in 1914 and thereafter, several reports of the disease were given in both livestock and man (Wright *et al*, 1953; Manson-Bahr, 1956; Oomen, 1976; Waghela, 1976; 1977). A serologic survey showing evidence of porcine brucellosis in Kenya was carried out by Waghela and Gathuma (1975). Another survey was carried out in North-Eastern Province which showed evidence of the disease in camels (Waghela *et al.,* 1978). The disease has since been reported in many parts of the country including Narok in humans (Muriuki *et al.,* 1997; Maichomo *et al.,* 1998;), Samburu, Kiambu and Kilifi in cattle (Kadohira *et a\.,* 1997), Nairobi and Naivasha in humans (Jumba *et al.,* 1996).

The prevalence of the disease in both man and livestock varies considerably depending on the livestock production system. It is higher in the pastoral production system where large numbers of livestock are kept and share close communal grazing fields and watering points. In addition, the animals are in close contact with the people (Kadohira *et al.,* 1997; McDermott and Arimi, 2002). In contrast, the disease has low prevalence in the intensive livestock production systems such as in zero-grazing due to low cattle to cattle contact.

Seropositivity to brucellosis has been shown to increase with the age of animals (Hellmann *et al.,* 1984; McDermott *et al.,* 1987; Kubuafor *et al.,* 2000). Sexually mature animals are very susceptible to brucellosis (Chukwu, 1987). Females have been shown to have increased chance of testing *Brucella* positive (Muma *et al,* 2006).

Brucella, the causal organism of brucellosis is a Gram negative, facultative intracellular bacterium. The organisms are cocci, coccobacilli or short rods measuring 0.5-0.7^im by 0.6-1.5}im, arranged singly and rarely in short chains. They are non-capsulated, non-spore forming and non-motile (Blood and Radostits, 1989).

There are six known species; *Brucella abortus, Brucella melitensis, Brucella suis, Brucella ovis, Brucella canis* and *Brucella neotomae.* All the above species except *Br. neotomae* are important pathogens. *Brucella abortus* is associated with cattle, *Brucella melitensis* with goats and sheep, *Brucella suis* with pigs, *Brucella ovis* with sheep, *Brucella canis* with dogs and *Brucella neotomae* with desert woodland rat (*Neotoma lepida)* (Chomel *et al.*, 1994).

Two new *Brucella* species, *Br. cetaceae* and *Br. pinnipediae* have recently been described from a wide variety of cetacean (dolphins, porpoises) and pinnipeds (seals) by Cloeckaert *et al.* (2001).

2.1.1 Sources of infection

The primary sources of contamination of the environment are the foetal membranes and fluids, and vaginal discharges which are expelled by infected females when they abort or at parturition. The *Brucella* organisms are also commonly shed in milk and semen (Radostits *et al*2000).

Livestock become infected after ingesting contaminated feed or water or licking an infected placenta, calf or foetus, or the genitalia of an infected cow soon after aborting or

calving, at which time *Brucella* organisms are present in the placenta lochia (Nicoletti, 1990). Other routes of less importance include inhalation via mucus membranes of the respiratory tract or through conjunctiva, and contact with contaminated material through intact and broken skin. Cows occasionally may be infected by coitus or when artificial insemination is done using infected semen. Calves may acquire infection in utero or they may become infected after ingesting infected colostrum or milk. Although some will rid themselves of the infection within a few months, others may remain infected for life and thus spread the disease at their subsequent parturitions (Anon, 1986).

Br. abortus has special affinity for the pregnant uterus because the placenta contains a high concentration of erythritol, a 4 carbon sugar-alcohol molecule which favours the multiplication of the organisms. *Brucella* organisms metabolize this sugar preferentially than other sugars and its presence in the placenta of ungulates explains the tropism of this pathogen for the reproductive organs and its capability to induce abortions (Jones and Hunt, 1983; Sangari *et al.,* 2006).

The mammary route also allows for escape of *Brucella* organisms into the environment. The infected animals develop *Brucella* induced mastitis and shed the organisms either continuously or intermittently throughout the lactation period and sometimes continue discharging the organisms in subsequent lactations. Cattle vaccinated before infection show a lower degree of *Brucella* excretion in milk than those not vaccinated (Radostits *et al***2000).**

The other mode of environmental contamination is through infected carcasses. Urine and faeces of some infected animals are less important sources of the bacterium. The fluid in hygromas caused by *brucella* infection may have large numbers of organism but since they are restricted to the lesion, they do not seem to play an important role in the spread of the disease (Anon, 1986).

Brucella survives in soil, water and manure for weeks or months depending on the material, temperature, humidity, pH and sun exposure. But they can remain viable in dead fetal material for even longer (Corbel, 2002). It may survive in aborted foetus in the shade for up to eight months, for two to three months in dry soil, three to four months in faeces, and eight months in liquid manure stored in tanks (Nicoletti, 1980; Anon, 1986).

2.1.2 Brucellosis in Animals

Domestic and wild animals are the reservoirs of *Brucella* organisms and man gets infected upon coming into contact with the infected animals and their products. In domestic animals, cattle, sheep, goats and pigs are mainly affected (Table 2.1).

In cattle, *Br. abortus* are usually the cause of brucellosis but *Br. melitensis* has also been implicated to cause abortion in cattle where they are kept in close association with infected sheep or goats (Radostits *et al.,* 2000).

Table 2.1: Most common diseases caused by *Brucella* in livestock species.

Brucella spp	**Livestock spp**	**Disease**
Br. abortus.	Cattle	Contagious abortion
Br. melitensis	Sheep and goats	Abortion and orchitis
Br. ovis	Sheep	Epididymitis and orchitis
Br. suis	Pigs	Abortion, stillbirth, sterility in sows and orchitis.

(source: Coetzer and Tustin, 2004).

Brucellosis in goats and sheep is mainly caused by *Br. melitensis* (Kabagambe *et al.,* 2001). The disease in these animals is similar epidemiologically to bovine brucellosis. Infection by *Br. suis* and *Br. abortus* has occasionally been found but is rare.

Brucellosis in pigs is caused by *Br. suis* and characterized by an initial bacteraemia followed by the production of chronic lesions in the bones and reproductive organs of both sexes (Radostits *et al.,* 2000).

Br. ovis is the most common cause of epididymitis in rams but rarely cause abortion in ewes and neonatal mortality in lambs. Classic brucellosis in sheep is caused by *Br. melitensis and* constitutes a public health problem. This infection is found in areas with mixed goat and sheep flocks. Sheep are more resistant to infection than goats and in areas of mixed flocks, fewer sheep than goats are found to be infected (Radostits *et al.,* 2000).

Br. amis causes epipidymitis and orchitis in male dog and metritis in bitches and it is a rare infection in humans (Radostits *et al*2000).

2.1.2.1 Clinical features of the disease

The establishment of the infection in the animal is influenced by the size of the infective dose, virulence of the bacteria and the immunity of the infected animal, age, sex and the reproductive status of the animal (Crawford *et al.,* 1991).

The clinical signs include the following: abortions which usually occur in the last trimester, weak, full-term neonates that often die shortly after birth, reduced milk yield, fever, infertility, mastitis especially in goats, in contrast to females of other species, where milk clots and small nodules appear on the mammary glands. Other signs are: retained placenta especially in goats, an acute to chronic uni- or bi-lateral orchitis, epipidymitis and seminal vesiculitis in males, scrotal circumference may be normal or severely increased (Godfroid *et* a/., 2004), uni- or bi-lateral hygromas especially of carpal joints in some chronically infected animals and progressive, erosive and non-suppurative arthritis of the stifle joints in some chronically infected animals (Anon, 1986; Alton, 1990; Grillo *et al.,* 1997; Elzer, 1998).

2.2 Highlights of Brucellosis

Brucellosis is an infectious zoonotic bacterial disease presenting a worldwide problem with significant public health and economic implications (Abela, 1999). Both domestic and wild animals act as reservoirs of *Brucella* pathogens for human infections (Baldi *et*

al., 1994). These bacteria are primarily passed among animals and cause disease in different vertebrates. The various *Brucella* species affect sheep, goats, cattle, deer, elk, pigs, dogs and several other animals including camels (Waghela *et al.,* 1978; Yagoub *et al.*, 1990; Bauman and Zeissin, 1992; Radostits *et al.,* 2000). Salem and Mohsen, (1997) demonstrated that fish could be considered as susceptible to brucellosis. Junaidu *et al.* (2006) has also demonstrated serological evidence of avian brucellosis in Nigeria. Humans become infected by coming into contact with animals or animal products contaminated by these bacteria or consuming contaminated animal products (Chomel *et al.,* 1994). In animals, the disease is characterized by abortion, retained afterbirth, orchitis, epididymitis, infertility, drop in milk yield and hygromas (Radostits *et al.,* 1989). In humans, the disease is characterized by undulating fever, sweating, headache, muscle pain, arthritis and neurological symptoms (Mousa *et al.,* 1986).

2.3 Culture characteristics of *Brucella*

Brucella species are strictly aerobic but many strains of *Br. abortus* when first cultured are unable to grow without the addition of 5-10% carbon dioxide. Temperature range for growth is 20-40°C and pH range is 6.6 to 7.4. All strains of *Brucella* grow best at 37°C in a medium enriched with animal serum and glucose. On clear solid medium, most *Brucella* strains grow slowly and after 24 hours, colonies are 0.5 to 1.0mm in diameter, raised, convex and with entire edges. Colonies are smooth and mucoid except for *Br. canis* and *Br. ovis* which are permanently rough (Jubb *et al.,* 1985).

2.4 Antigenic structure

Brucella organisms have a closely related antigenic structure, which makes their differentiation in serologic studies difficult. They localize and proliferate within the cytoplasm of monocytes and reticulo-endothelial cells (Jubb *et al.*, 1985) and are thus protected from host defence mechanisms. In all the smooth strains, the dominant surface antigen is a lipopolysaccharide-0 chain which, depending on the three dimensional structure, forms A, M or C epitopes. These are common to all smooth species but the distribution of A and M depends on biovar (Corbel, 1997). Rough strains do not produce the lipopolysaccharide-0 chain but have a common R epitope. The lipopolysaccharide has endotoxin activity and elicits antibody-mediated protection (Corbel, 1997). More complete immunity is dependent on cell-mediated, particularly cytotoxic responses elicited by ribosomal and other proteins.

2.5 Diagnosis in animals

Diagnostic tests for brucellosis are subdivided into three groups;

i. Tests that demonstrate *Brucella* organisms,
ii. Tests that detect immunoglobulins.
iii. Those that depend on allergic reactions (Anon, 1986).

The best approach in the diagnosis of brucellosis is a combination of epidemiology, serology, clinical and bacteriologic evidence (Ramon and Ignacio, 1989). The presumptive diagnosis based on clinical history of abortion, retained placenta in the

females and lesions in the seminal vesicles and testis in the male must be sustained with demonstration of the organism and or specific antibodies in the body fluid for making a confirmatory diagnosis of *Brucella* infection (Chakrabarti, 1993). Laboratory diagnosis relies most on serological test.

2.5.1 Laboratory diagnosis

Brucellosis is confirmed by isolating the organism from blood or other tissue samples and by serological tests. In animals, culture is attempted from abortion material, placenta, milk, semen or from samples of lymphoid tissue, mammary gland, uterus or testis collected at postmortem (Jubb *et al.*, 1985).

2.5.1.1 Direct Microscopic Examination

Using Stamp's modification of Ziehl-Neelsen stain, *Brucella* organisms stain red against a blue background in tissue sections and smears. However, this colour change reaction is not specific as *Coxiella burnetii, Chlamyclophila arbotus* and *Norcardia* species are also weakly acid fast. *Norcardia* spp can be differentiated from these organisms on morphological grounds, but it is very difficult to differentiate *C. abortus, C. burnetii* from *Brucella* spp. beyond any doubt (Jubb *et al.,* 1985).

2.5.1.2 Culture and typing

Blood culture is attempted at all phases of infection, taking at least 10ml of blood on each occasion and adding 5ml to each of the two blood culture bottles containing

glucose-serum broth. One of the bottles is incubated in an atmosphere containing 10% carbon dioxide (Radostits *et al.,* 2000).

Other specimens include the following; foetal membranes, lungs, and spleen, stomach, liver and spleen of aborted foetus and full-term calves; and from live cows uterine discharge, milk or colostrum. Supramammary lymph nodes are preferred for isolation of *Br. abortus* from animals that have been slaughtered and 90% recovery rate from infected animals may be achieved (Godfroid *et al.,* 2004). In order to get valuable epidemiological information, isolated *Brucella* have to be typed (species and biovar) (Anon, 1986).

Blood culture results for acute primary infection is highly sensitive, but in individuals with occupational exposure or with symptoms of acute, persistent and often unspecific infection as observed in endemic areas, the test gives poor results (Serra and Vinas, 2004). Blood cultures should be retained for 6-8 weeks before being discarded as negative.

2.5.1.3 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction based laboratory tests have been proposed but they cannot be considered as a routine diagnostic method yet (Serra and Vinas, 2004). The PCR with primers specific for the omp2, omp25 and rrs-rrl genes can detect *Brucella* specifically and also give an indication of species and biovar (Cloeckaert et al., 1995)

hut is poorly suited for use in a general diagnostic laboratory (Fredricks and Relman, 1999).

2.5.1.4 Serological tests

The limitations of blood culture and PCR based laboratory tests make serological tests the most useful tool for laboratory diagnosis of *Brucella* infection (Serra and Vinas, 2004). Most of the serological tests for the diagnosis of smooth *Brucella* spp. infections *(Br. melitensis, Br. abortus* and *Br. suis*) have been developed to detect antibodies directed against antigens (mainly A and/ or M epitopes) associated with the smooth lipopolysaccharide (S-LPS) and are shared by all the naturally occurring biovars of *Br. abortus, Br. melitensis* and *Br. suis* (Godfroid *et al*., 2004).

i. Rose Bengal Plate Test (RBPT)

An antigen stained with Rose Bengal and buffered at pH 3.65 is mixed in equal volumes (30^1) with test serum and shaken for four minutes. Any degree of agglutination is an indication of a positive test. The test is simple, inexpensive, sensitive and widely used as a screening test. False negative results are rare and are usually obtained during the more chronic stages of the disease. This test is prescribed for international trade in cattle by the OIE (Sutherland *et al.,* 1986; Nielsen *et al*., 1996; Kadohira *et al*1997; Anon, 2000).

Serum Tube Agglutination Test (SAT)

The test is used as a screening test for eradication of brucellosis in some countries where it is used as a supplementary test for indicating levels of immunoglobulin M, the predominant immunoglobulin after vaccination with strain 19 vaccines (Alton *et al.y,* 1975). The sensitivity is rather low and lacks specificity (Godfroid *et al.,* 2004). The SAT has the advantage of detecting the combined IgM, IgA and IgG antibody levels in serum, but its diagnostic specificity is poor, especially when the titres are low. Cross reactions with other gram-negative bacteria have been observed, and diagnostic end-point agglutination titre has not been satisfactorily established (Lucero *et al.,* 1999).

2-Mercaptoethanol Test

This is an agglutination test which utilizes 2-mercaptoethanol (2-ME) for inactivating immunoglobulin M and A molecules in the serum. The test indicates the presence of IgG, an indication of persistent infection as observed in chronic infection (Alton *et al.,* 1975). The test is also used in determining the adequacy of antibiotic therapy where a negative 2ME test is strong evidence against a diagnosis of chronic brucellosis (Buchanan and Faber, 1980). The test has low sensitivity of 59% (Dohoo *et al.,* 1986).

Complement Fixation Test

In this test, the titres do not wane as the disease becomes chronic and therefore the IgG and IgA which remains present in the serum can still be detected by Complement Fixation Test (CFT). The test is therefore used for the diagnosis of both acute and chronic infections. The test has several weaknesses, such as the occurrence of anticomplement activity, the need to use a highly labile reagent (such as complement), failure of the test to detect a CFT response in the early stage of the disease, and the technical demands (Lucero *et al.*, 1999).

Indirect Enzyme -Linked Immunosorbent Assay (iELISA)

Indirect enzyme-linked immunosorbent assay (iELISA) is more sensitive in detecting antibodies to *Brucella* spp. than are RBPT, SAT and CFT, but great care must be exercised in animals vaccinated with strain 19 vaccine (Sutherland, 1984; Nielsen, 2002). It is also rapid to perform in the laboratory and can be standardized with ease (Portanti *et al.*, 2006).

Competitive Enzyme -Linked Immunosorbent Assay (cELISA)

This test is a multispecies assay which appears to be capable of differentiating vaccinal and cross-reacting antibodies from antibodies elicited by field infection in livestock (Lucero *et al.*, 1999). The basis of this test is the use of a selected monoclonal antibody (Mab) that competes with low affinity antibody. The competitive enzyme linked immunosorbent assay (cELISA) using a Mab specific

for one of the epitopes of *Br. abortus* O-PS has been shown to have higher specificity than iELISA (Sutherland, 1984). The test is more specific, eliminates cross reaction in serological tests with *Yersinia enterocolitica* infection and vaccination with strain 19. The test is prescribed as an alternative test for international trade in cattle by the OIE (Sutherland *et al.,* 1986; Nielsen *et* al., 1996). ELISA has been shown to be superior to RBPT and CFT (I Iornitzky and Searson, 1986) and is also capable of successfully differentiating acute brucellosis and chronic brucellosis (Araj *et al*., 1986, Lulu *et al.,* 1988).

vii. Coombs (antihuman-globulin) Test

It is an agglutination test utilizing Coomb's reagent, an antiserum specific against either globulin or whole serum. It is very sensitive and detects exposed individuals such as veterinarians and laboratory workers who may be symptomless. At a cut-off point of 1/320, the test has a sensitivity of 92% and specificity of 100% (Martin Moreno *et*«/., 1992).

viii. Milk Ring Test (MRT)

The milk ring test is used to detect antibodies in milk. The test depends on two reactions: (i) fat globules in milk are aggregated by milk antibodies (fat-globule agglutinins); and (ii) stained *Brucella* antigens are added to the milk and will be agglutinated by the *Brucella* antibody in the fat globule and then rise to form a coloured cream layer at the top (Anon, 1986). This is a sensitive screening test

used on bulk milk samples either to detect infected animals on a herd basis or to monitor clean herds.

False positive results occur under the following circumstances:-

a) a high prevalence of mastitis,
b) a high proportion of cows in early or late lactation,
c) recent (within three to four months) vaccination with strain 19 vaccine,
d) souring of milk.

ix. Brucellin Allergic Test.

Hypersensitivity to *Brucella* antigens is acquired following exposure to infection, vaccination or following exposure to the organisms or killed antigens in the laboratory. The test is considered to have low sensitivity at the animal level but the specificity exceeding 99%, and thus a useful method of identifying infected herds rather than individual animals (Alton *et al.,* 1988). Experimental studies have shown that it is the only test that is able to discriminate between *Y. enterocolitica* 0:9 and *B. abortus* infections beyond any doubt (Godfroid *et al.,* 2002). The chief value of the test is for epidemiological purposes and is now a recommended herd test by the OIE (Anon, 2000).

2.6 Treatment of brucellosis in animals

Treatment of animal brucellosis is not normally done and when attempted, it is frequently not successful due to the intracellular sequestration of the organisms in the lymph nodes, the mammary glands and reproductive organs (Radostits *et al.,* 2000).

Treatment with antibiotics has been attempted with varying results; long acting oxytetracycline (20mg/kg, IM) alone or combined with streptomycin (25mg/kg IM or IV) has been used (Nicoletti *et al.,* 1985). In addition to the above drugs, intramammary infusions have been used (Nicoletti *et al.,* 1989). Rwadan *et cil*., (1992) suggested that treatment of *Br. melitensis* infection in sheep and goats with long acting oxytetracycline 25mg/kg IM every two days for four weeks combined with streptomycin 20mg/kg IM every two days for two weeks was the most practical, effective and least expensive regimen for eliminating *Brucella* in sheep and goats. In rams, treatment is economically practical only in very valuable rams to save the genetic pool and must be instituted early before irreparable damage to the epididymis has occurred (Radostits *et al.,* 2000). However, *Brucella* spp. may undergo L-transformation when exposed to certain antibiotics, such as penicillin and oxytetracycline and these cell wall deficient forms prevent serological reaction (Anon, 1986; Banai *et al.,* 2002).

Antibiotic use is discouraged in recently vaccinated animals because they tend to interfere with the development of immunity (Smith *et al.,* 1983).

2.7 **Brucellosis in Humans**

Br. melitensis is the most common cause of human illness and is very pathogenic (Anon, 1986). *Br. abortus, Br. suis* and *Br. cams* are also relatively common causes (FAO 2003). Animals are almost exclusive source of brucellosis infection for humans (Alton *et al.,* 1988; Schelling *et al.,* 2003). Infection is through direct contact with infected animals especially when aborting, animal carcasses and indirectly through consumption of unpasteurized milk and milk products or inadequately cooked meat from infected animals, a common practice amongst the pastoral communities (Omore *et al.,* 1999; Rust, 2004). Laboratory workers are at risk by coming in direct contact with the organisms. The respiratory tract and the conjunctiva may also act as portals of entry by the bacteria (Anon, 1986). Epidemiological evidence has shown that at least 90% of human *Brucella* infection can be attributed to direct contact with infected livestock and to consumption of contaminated raw milk or raw milk products (Baron and Finegold, 1990) and the number of human cases is directly related to the prevalence of the infection in animals (Al-Ani *et al.,* 2004). Aerosal infection has been reported. *Brucella* organisms can also penetrate through damaged skin or through the eye (conjunctiva).

Brucellosis is an occupational disease with the following groups being at risk; herdsmen, abattoir workers, veterinarians, dairy industry professionals, microbiologic laboratory personnel and meat inspectors (Kubuafor *et al.,* 2000).

2.7.1 Transmission

Transmission occurs through direct contact with infected animals, animal carcasses, aborted material or placentas, vaginal discharges, blood and urine. Food-borne infection occurs following ingestion of raw milk and other dairy products such as cheese, cream, butter, chocolate and yoghurt if prepared from unpasteurized milk (CDC, 1975). Transmission rarely occurs from eating raw meat from infected animals (FAO, 2003). In addition, transmission can also occur through contact with the organisms in the laboratories as well as accidental inoculation with live vaccines such as *Br. melitensis* Rev.l and *Br. abortus* strain 19 vaccine (Alton, 1985). The humans most at risk are those in areas where the infection in animals has not been controlled, consume raw milk and live in poor hygienic conditions (Anon, 1986; Amin *et al.,* 2001). Infection can occur through inhalation of contaminated aerosols and dust. Transmission can also occur by blood transfusion or organ transplant (Radostits *et al.,* 2000). The only sure way of containing the disease in humans is to control and prevent the disease in animal reservoirs (Zhunushov and Kim, 1991).

Human-to-human transmission is limited but has been recorded where an infant suckled an infected mother (Varon *et al.,* 1990). Venereal transmission has also been reported between a laboratory worker and his spouse, and *Br. melitensis* abscesses in a woman's breast serve as a source of infection for the infant (Olsen *et al.,* 2004).

2.7.2 Clinical features

Brucellosis is a multi-system disease that may present with a broad spectrum of clinical manifestations (Yetkina *et al*., 2006). The incubation period is generally 1-2 months, and thereafter the infection may remain latent, sub-clinical or give rise to infections of varying intensity and duration. In the acute form (less than 8 weeks from onset of illness), the clinical signs include non-specific and 'flu-like' symptoms including fever, sweats, headache, chills, malaise, anorexia, myalgia, weight loss and profound weakness. In the undulant form (less than 1 year from onset of illness), symptoms include intermittent fever, malaise, arthritis, stiffness of the neck and epididymo-orchitis in males. Other signs include hepatosplenomegaly, hepatomegaly and splenomegaly. The characteristic intermittent waves of elevated temperature are usually seen in long standing untreated cases (Corbel, 2002).

Neurobrucellosis may occur in up to 5% of the cases. The signs include confusion, gait disorders, depression, insomnia and paralysis (Yetkina *et al*., 2006).

Brucellosis can last for up to several months resulting in a debilitating disease. The case fatality rate is very low except for cases of *Br. melitensis* which causes endocarditis (Alemayehu, 1981). Chronic sequelae of the disease may include hepatic disease, endocarditis, colitis and meningitis (Olsen *et al.,* 2004).

It has been shown that there is a high incidence of first and second trimester spontaneous abortion among women with active brucellosis (Khan *et al.,* 2001).

Papular to pustular skin rashes which are sometimes evident on the arms of veterinarians following obstetric procedures have been attributed to allergy to *Brucella,* but sensitivity to other pathogens including *Salmonella typhimurium* and *Listeria monocytogenes* have been incriminated (Anon, 1986).

2.7.3 Diagnosis

Brucellosis in humans presents non specific signs which are shared by other flu-like diseases which include malaria, typhoid, streptococcal infections and rheumatism (Hendricks *et al.,* 1995; Muriuki *et al.,* 1997; Mutanda, 1998; Maichomo *et al.,* 2000) and this makes diagnosis difficult. In man, disease diagnosis is largely based on clinical symptoms of fever, joint pains combined with epidemiological data or risk assessment such as contact with livestock or consumption of unpasteurized dairy products (Tsertsvadze *et al.,* 2006). But accurate diagnosis necessitates the use of specific tests mainly culture and serological tests (Araj and Azzam, 1996; Lucero *et al.,* 1999). Isolation by culture of citrated blood on selective media or inoculation into guinea pig is recommended (Alton *et al.,* 1975) but not always possible. In individuals with previous contact with the microorganism or occupational exposure and symptoms of acute, persistent and often unspecific infection as is common in endemic areas, blood culture gives poor results (Serra and Vinas, 2004). Serological tests therefore provide the most common and routine method for diagnosis in the laboratory. The tests used include Serum Agglutination Test, Rose Bengal Plate Test, Complement Fixation Test (Alton *et al.,* 1975). Others are Enzyme-Linked Immunosorbent Assay (ELISA) and the

Polymerase Chain Reaction (PCR). PCR can also be used to detect *Brucella* specifically and even to the biovar level (Corbel, 2002).

2.7.4 Treatment of brucellosis in humans

Treatment of brucellosis is still far from ideal (Grushina *et al.,* 2006). *Brucella* infections respond to a combination of streptomycin (lg/day) or gentamycin and tetracycline or rifampicin (600 to 900mg/day and doxycycline (200mg/day). Tetracycline alone is often adequate in mild cases. Treatment should be continued for at least six weeks (Lucero *et al.,* 1999; Corbel, 2002). Co-trimoxazole and rifampicin can be used in children. In endocarditis and neurobrucellosis, a combination of aminoglycoside, tetracycline and rifampicin is recommended (Corbel, 2002). But effective treatment requires early diagnosis (Al Dahouk *et al.,* 2003).

2.7.5 Prevention and control of brucellosis in both animals and humans

The control of the disease is based on testing and slaughter of infected animals, hygienic measures and vaccination. Surveillance is very important once control or eradication procedures have been initiated. This is geared towards eliminating the disease in animals (FAO, 2003). This can be achieved by a combination of vaccination of all breeding animals to reduce the risks of abortion and raise herd immunity, followed by elimination of infected animals or herds by segregation and slaughter.

Methods of prevention include health education to reduce occupational and foodborne risks, including pasteurization of all dairy products. However, education campaigns have never resulted in fully eliminating the risks of infection (Corbel, 2002; FAO, 2003).

During the Brucellosis control program, mass testing is carried out where positive reactors are removed from the herd and the negative ones are retested two or three times after 1-2 months so that the negative ones are declared *Brucella*-free and then separated immediately and protected from infection through improved hygiene. In herds where reactor animals are many and slaughter cannot be carried out, the reactors are completely separated and followed by rigorous cleansing and disinfection, and disposal of infective material. Aborting or parturient animals should be isolated from 4 days prior to and 14 days after parturition (Arthur *et al*1989). Other measures capable of reducing the rate of infection in a herd are:

i. Improved hygiene at milking to prevent spread from udder through milker's hands.
ii. Providing the best accommodation possible where animals are housed.
iii. Weaning the newborns at the earliest possible time and rearing them in a *Brucella* free environment.

In a region or country, eradication is only feasible if the prevalence is less than 2% and this is implemented through a test and slaughter program. This is expensive and requires a strong political, financial, technical and social backing (Muriuki, 1994).

Vaccination is carried out using vaccines prepared from strains of *Br. abortus,* namely; strain 19, a smooth strain used as a live attenuated vaccine, strain 45/20 and 1138 as a rough killed vaccine and more recently, strain RB51 as a rough live attenuated vaccine (Godfroid *et al.,* 2004). Rev 1 strain is prepared from *Br. melitensis* and is commonly used in small ruminants (Verger and Plommet, 1985). Vaccination does not eliminate infection and is not of any value from a public health point of view as consumers of raw animal products remain at risk. It is only of value in reducing losses arising from abortions. Live vaccines provide more prolonged immunity compared to inactivated vaccines.

In pastoral areas, control and eradication measures for a disease such as brucellosis is difficult to implement because of the communal grazing, indiscriminate herd expansion, nomadism, low levels of hygiene and poverty. The area lacks adequate clean water. In addition, there is very close association between animals and man and therefore easy transmission of the disease between man and animals (Muriuki, 1994).

Human vaccination is not recommended because effective and non-reactogenic vaccines are not available despite considerable effort (Al Dahouk *et al.,* 2003; Smits and Cutler, 2004).

3 MATERIALS AND METHODS

3.1 Study Area

The study was undertaken in northern Turkana District, Rift Valley Province of Kenya, between October 2006 and February 2007. Turkana District is located in the northwestern part of Kenya, bordering with Uganda to the west, Sudan to the northwest and Ethiopia to the north. Within Kenya, the District borders Baringo and West Pokot to the south, Samburu District to the southeast and Marsabit District to the east (Fig. 3.1). It is situated between longitudes 34°0' and 36°40' east, and between latitudes 10°30' and 5°30' north (Turkana District Development Plan, 2002-2008)

Turkana District, the largest in Kenya, has an approximate area of 77,000km^2 with 17 divisions, 56 locations and 156 sub-locations. The human population is 497,780 with an average density of 7 persons per km^{-9} with the highest density being 29 persons per km^{-9} in Kakuma and the lowest being one person per knr in Kibish (Turkana District Development Plan, 2002 -2008). The District falls in the region classified as Arid and Semi-Arid Lands (ASAL) receiving an average annual rainfall of 300-400mm, which is erratic and unreliable. The temperatures range between 24°C and 38°C. The vegetation is predominantly deciduous annual grassland with scattered dwarf shrubs or trees. The area has few seasonal rivers most of which drain into the Lotikipi plains. There are some springs along the foot of the hilly ranges. Water sources for majority of the people are pools during the rains, traditional shallow wells dug into the dry river beds and water pans. Other water sources include bore holes and rock water catchments (Arid Lands

Resource Management Project II Drought Monthly Bulletin for January, 2007: Turkana District). Out of the total 80,921 households in the District, only 23,000 have access to potable water and the average distance to the nearest potable water point is 10 km (Turkana District Development Plan, 2002-2008).

The economy of the District depends mainly on livestock. Majority of the population in the District practice pure pastoralism (64%) while others (16%) are agro-pastoralists, mainly in the southern and western part of the District. The rest comprise the fisher folk and urban and peri-urban population who fall out from pastoralism.

Turkana District is regarded as one of the poorest districts in the country. According to the 1997 welfare monitoring survey (WMS II), it recorded an overall poverty of 74%, food poverty of 81%, and hard-core poverty of 62%. In absolute numbers this is equivalent to 333,636 overall poor, 365,196 food poor and 279,533 hard-core poor out of a total population of 485,526. The major causes of poverty in the District are harsh topography, harsh climatic conditions and prevalence of livestock diseases among others (Turkana District Development Plan, 2002-2008). Livestock population is estimated at 197,700 head of cattle, 2,021,000 goats, 1,054,400 sheep, 35,160 donkeys, 172,400 camels and 10,368 poultry (DVO Annual Report, 2004).

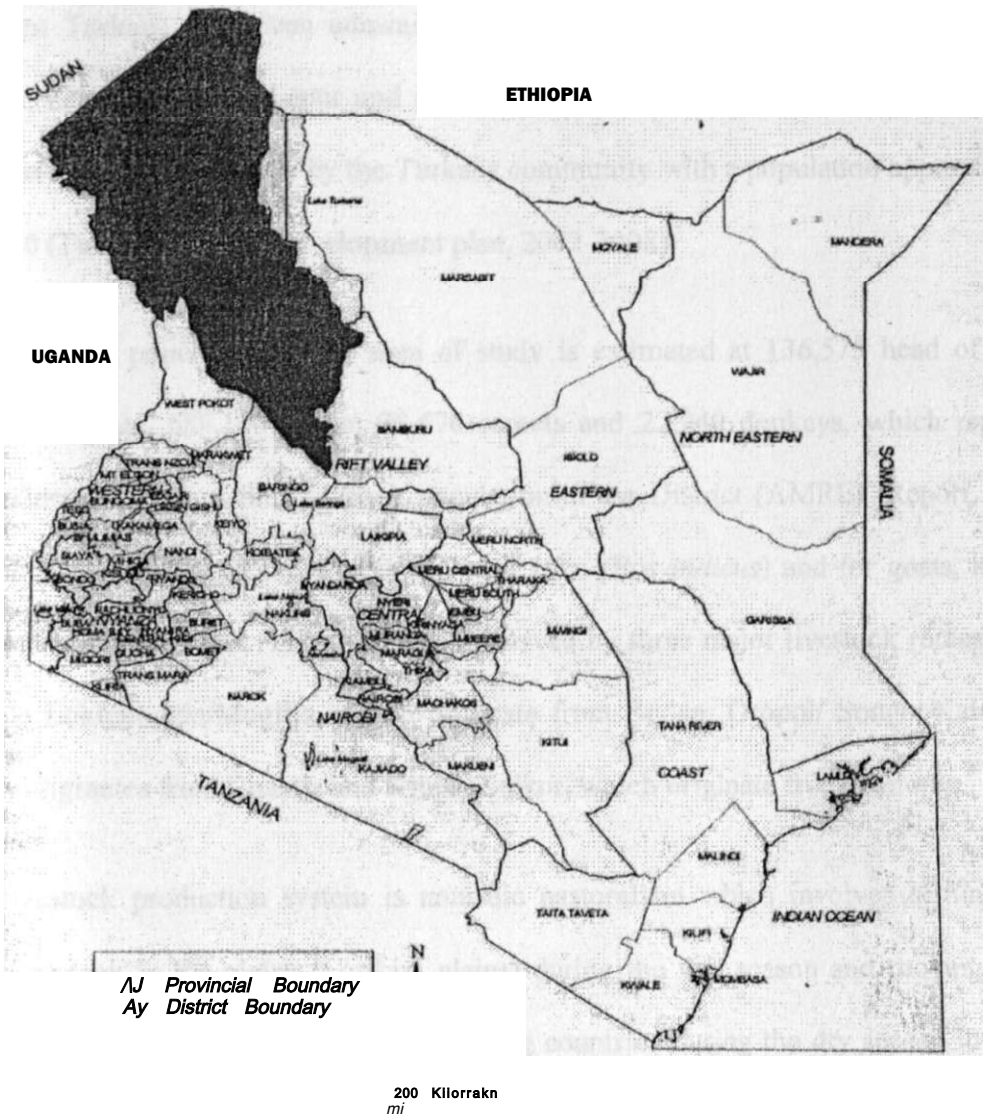


Figure 3.1: Map of Kenya showing the position of Turkana District in Kenya (Shaded black). Source: Turkana District Development Plan, 2002 -2008.

Northern Turkana has seven administrative divisions namely; Lokichoggio, Kakuma, Oropoi, Kibish, Kaaling, Lapur and Lokitaung (Fig.3.2). The area is about 20,000 km and inhabited predominantly by the Turkana community with a population approximately 233,520 (Turkana District Development plan, 2002-2008).

The livestock population in the area of study is estimated at 136,575 head of cattle, 1,379,000 goats, 689,100 sheep, 98,670 camels and 22,940 donkeys, which represent approximately 70% of the livestock population in the District (AMREF Report, 2004). The cattle breed kept in the area is the local zebu *(Bos indicus)* and for goats, it is the Small East African goat. The study area is served by three major livestock routes which include Lokichoggio/Mogilla, which originate from Sudan, Oropoi/ Songot/Kalobeyei, which originates from Uganda and Kibish/Kaikor, which originate from Ethiopia.

The livestock production system is nomadic pastoralism which involves settling with their livestock in the plains (Lotikipi plains) during the wet season and moving to the high mountain ranges and to the neighbouring countries during the dry season in search of pastures and water for their livestock. Every month, they move further up into hills with their cattle, followed by sheep, and goats that browse the areas already grazed by cattle, until the rains return and they move back to the plains. Insecurity is a major problem both in the plains and the hills. This comes in the form of cattle rustling between all the neighbouring communities of the region, the Toposa of southern Sudan, Dong'iro and Merile of Ethiopia and the Jie of Uganda. This practice puts large tracts of land along the borders out of use and limits mobility on the remaining land (Barret, 1998).

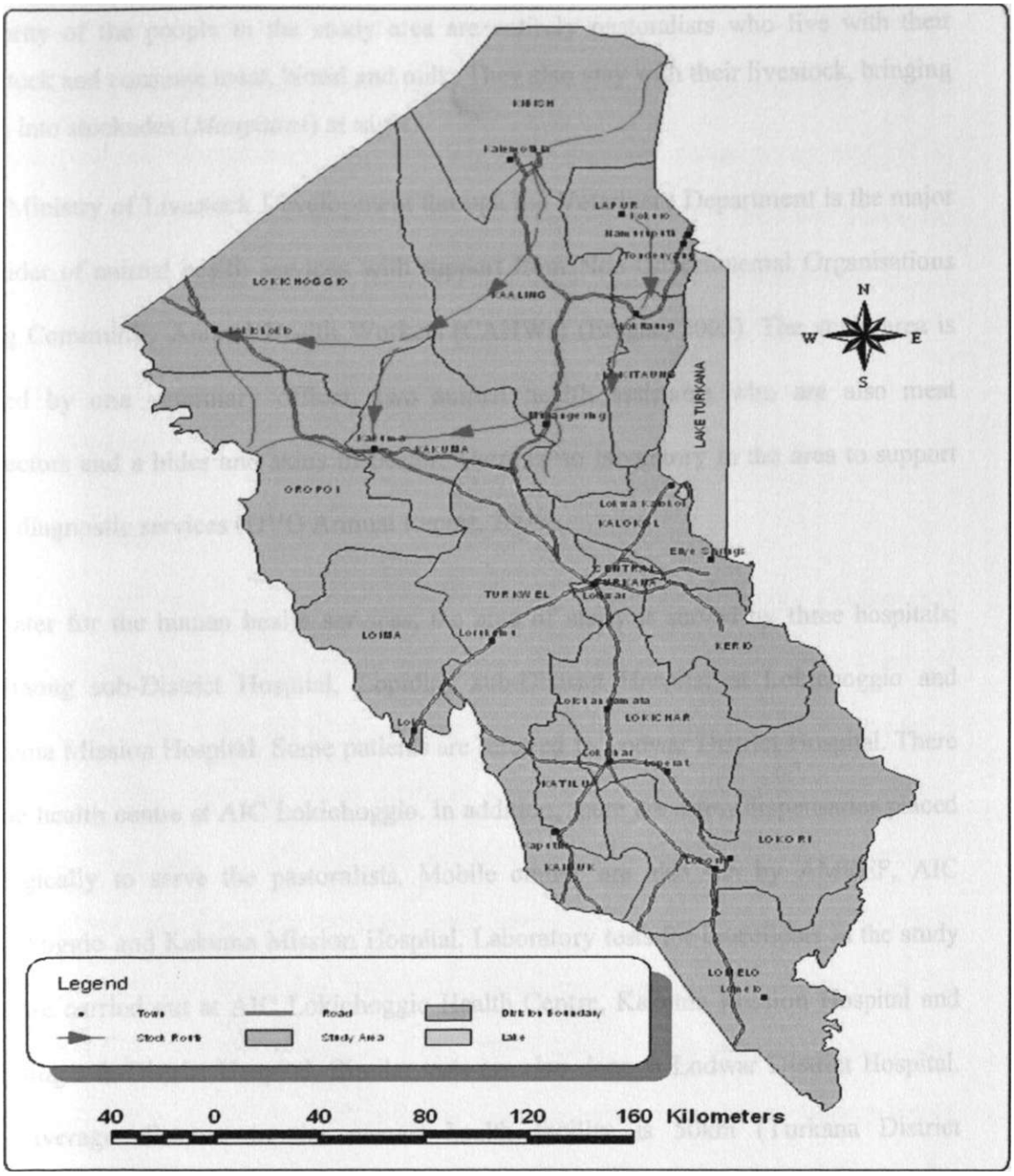


Figure 3.2: Map of Turkana District showing the study area and the livestock routes.

Majority of the people in the study area are entirely pastoralists who live with their livestock and consume meat, blood and milk. They also stay with their livestock, bringing them into stockades (*Manyattas*) at night.

The Ministry of Livestock Development through the Veterinary Department is the major provider of animal health services with support from Non-Governmental Organisations using Community Animal Health Workers (CAHWs) (Eregae, 2003). The study area is served by one veterinary officer, two animal health assistants who are also meat inspectors and a hides and skins inspector. There is no laboratory in the area to support field diagnostic services (DVO Annual Report, 2006).

To cater for the human health services, the area of study is served by three hospitals; Lokitaung sub-District Hospital, Lopiding sub-District Hospital at Lokichoggio and Kakuma Mission Hospital. Some patients are referred to Lodwar District Hospital. There is one health centre at AIC Lokichoggio. In addition, there are a few dispensaries placed strategically to serve the pastoralists. Mobile clinics are also run by AMREF, AIC Lokichoggio and Kakuma Mission Hospital. Laboratory tests for brucellosis in the study area are carried out at AIC Lokichoggio Health Centre, Kakuma Mission Hospital and Lopiding sub-District Hospital. Similar tests are also done at Lodwar District Hospital. The average distance to the nearest health facility is 50km (Turkana District Development Plan, 2002-2008).

The Turkana community has a social organizational structure which is hierarchical starting with the territorial clans *(citeker),* which are subdivided into livestock camps or

grazing units (*cuiakaar)* and then families *(awi)* which comprises of a man, his wives and children. Each livestock camp or *adakaar* consists of between twenty to fifty families depending on the security situation in the area. The livestock camps move together as they search for pastures and water for their livestock for security reasons. There are thirteen livestock camps (*adakaars*) in the study area which are found in different parts of the region (AMREF Report, 2004).

3.2 Sampling

3.2.1 Livestock.

The study area was stratified into three regions, namely, Mogilla, Oropoi and Kibish/Kakuma. Stratification was based on how they are served by the three livestock routes (Figure 3.2). A full list of the thirteen *adakaars* in the region was obtained from the AMREF office data base. Three *adakaars* were selected conveniently in each region (Table 3.1). The basis of selection was accessibility due to terrain or floods, logistical considerations and the security situation. The approximate number of households in each selected *adakaar* was established through the community agents and was verified at the office of the chief at the respective areas. The *adakaars* were accessed through the assistance of the chiefs. In each selected *adakaar,* five households were selected randomly by assigning the head of each household present a 'yes' or 'no' card. One who picked a 'y^{es}' card qualified to participate in the study while a 'no' card meant not participating. Each household contributed sample sizes of cattle and goats proportional to the herd and flock size. In this study, cattle and goats of one year and above were selected

using systematic random sampling at a household level. The selection was done without paying attention to the sex of the animal. Due to disparities in population distribution, the samples were collected in such a way that 40% of the sample size was from Mogilla and Oropoi regions while the remaining 60% was obtained from Kibish/Kakuma region which is relatively large and both human and livestock populations high.

3.2.2 Humans.

The study team visited the selected *adakaars* and through a meeting with community leaders, the number of households in the *adakaar* was verified. The number of people selected in each *adakaar* was proportional to the *adakaar* size. For each household, a representative picked a 'yes' or 'no' card where a 'yes' card meant the person becomes part of the study. Any member who objected was replaced randomly by another member from the same settlement. However, attempts were made to ensure a high level of cooperation. In a situation where there was insufficient number of individuals, the exercise was repeated in the neighbouring settlement. In addition to the nine *adakaars,* there was a group which comprises those people that have settled at the peri-urban centres. This group of people does not ascribe to a specific *adakaar* and comprises those that have fallen out of pastoralism. This constituted the settled/semi-settled group. They normally keep relatively few animals compared to the pastoralists, usually goats. This group of people was similarly selected as for the *adakaars.* For each selected household, a questionnaire was administered to the head of the household and thereafter, any member of the household irrespective of age and gender volunteered for serum

collection. Care was taken to ensure only one member per household volunteered. For each individual below the age of 18 years, the consent of their parents was sought. A total of 174 people enrolled in the study. They were aged between 9 and 69 years with a mean of 36; 44 were females and 130 were males. Figure 3.3 shows the areas where the samples were collected.

Table 3.1 The ***adakaars*** selected in the study area per strata

REGION	***Adakaars***
Oropoi	1. Edoe 2. Apamulele 3. Ng'itoroboi.
Mogilla	4. Ng'iwoiyasike. 5. Ng'apurusio. 6. Ng'inyamakidiok
Kibish/Kakuma	7. Ikong. 8. Eipa. 9. Manaa .

3.3 Data Collection

The study team visited the community in the selected ***adakaars.*** A brief meeting was held with the community members where the study team introduced itself and the objectives of the visit highlighted. During the meeting, households were randomly selected to participate in the study. The disease under study was introduced and through group discussions, knowledge gaps regarding the disease were identified. Semi-structured questionnaires were designed to elicit information on the risk factors for brucellosis in both livestock and humans. These questionnaires were administered by the investigator via personal interviews with assistance of interpreter to household heads (Appendices I and II). Correct interpretation was ensured by virtue of the principle investigator's basic understanding of the Turkana language. The information collected included the following:-

A. Brucellosis in livestock

i. Management (grazing and watering) system.

ii. Introduction of new stock into the herd in the last one year.

iii. The livestock owner's level of awareness or knowledge about brucellosis.

iv. Frequency of contact of the livestock owners with livestock extension officers.

B. Brucellosis in Humans.

i. Close association with livestock, through sharing of compound, houses or water sources.

ii. Consumption of unprocessed or under-processed livestock products such as raw milk, raw blood and undercooked meat or that which is not roasted well.

iii. Level of awareness or knowledge about brucellosis. In this case, the respondents were classified into three groups; those that knew the manifestation of the disease in both livestock and humans were classified as completely aware; those that knew the manifestation of the disease in livestock only or in humans only were classified as partially aware and those that did not know the manifestation of the disease in both livestock and humans were classified as completely not aware.

For each person interviewed, the age, sex and *adakaar* was recorded.

Two more questionnaires were administered to the District Veterinary Officer (Appendix III) and the District Medical Officer of Health (Appendix IV) to elicit information regarding the disease occurrence in the district, symptoms/clinical signs, management, history of vaccination in livestock and trend of the disease in the last five years for livestock and humans, respectively.

Laboratory records at the four health facilities, Lodwar District Hospital, Kakuma Mission Hospital, AMREF Clinic (currently closed) and AIC Lokichoggio Health Centre which carry out laboratory diagnosis of brucellosis were taken to establish the trend of brucellosis in humans between 2001 and 2006.

3.4 Sample size determination

The sample sizes for cattle, goats and humans for bleeding were determined using the formula in Martin *et al.* (1987):

$$n = \frac{pq}{Z_s^2}$$

Where,

n is the required sample size.

Z_a=l .96, the normal deviate at 5% level of significance.

p is the estimated prevalence of Brucellosis. q=l -p.

L is the precision of the estimate, 5%.

Cattle: Using an estimated prevalence of 15% (Kadohira *et al;* 1997):

$$n = \frac{1.96^2 \times 0.15 \times 0.85}{(0.05)^2}$$

= 196 head of cattle.

Goats: the prevalence of the disease in goats is not known and therefore using the estimated prevalence (p) of 50%,

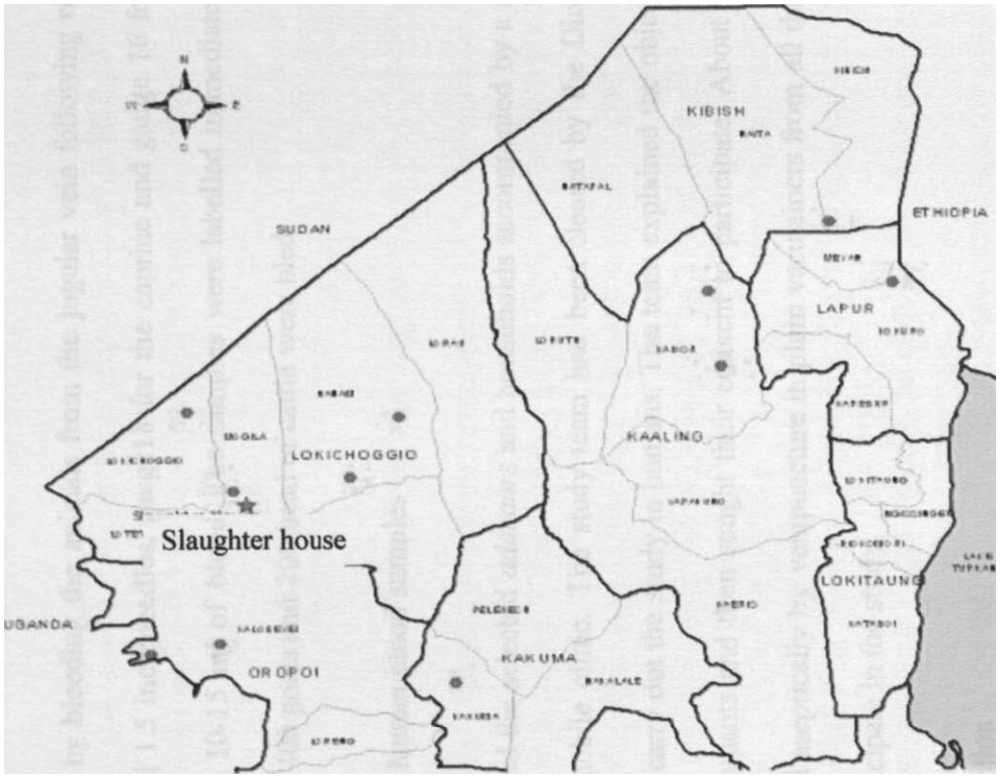
$$n = \frac{1.96^2 \times 0.5 \times 0.5}{(0.05)^2}$$

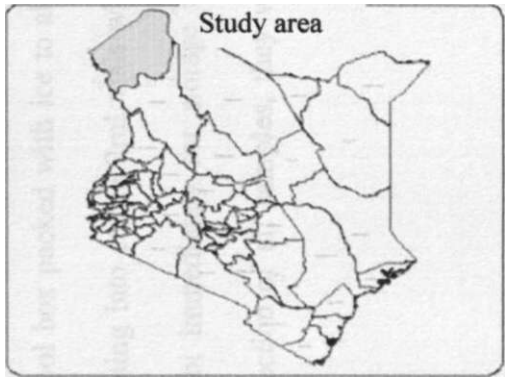
= 384 goats.

Humans: using an estimated prevalence of 12% (Maichomo, 1997),

$$n = \frac{(1.96)^2 \times 0.12 \times 0.88}{(0.05)^2}$$

= 162.





LEGEND

- Sample points

Lake Turkana

Figure 3.3 Map to show where samples were collected.

3.5 Serum Collection

3.5.1 Collection of cattle and goat blood samples

Blood was collected by bleeding the animals from the jugular vein following restraint. Plain vacutainers and 1.5 inch needles, gaugel8 for the caprine and gauge 16 for cattle were used to collect 10-15 ml of blood. The samples were labelled immediately after collection. A total of 400 goats and 200 head of cattle were bled.

3.5.2 Collection of human blood samples

The study team visited the selected ***adakaars*** and households accompanied by a member from the AMREF mobile clinic. The study team had been cleared by the Director of Medical Services to carry out the study in humans. The team explained the objectives of the study to the occupants and then sought their consent to participate. About 10ml of blood were collected aseptically by venipuncture in plain vacutainers from all those who gave consent to participate in the study.

3.5.3 Serum separation

The blood samples were left to stand overnight in a cool box packed with ice to allow serum separation. Serum was then harvested by decanting into sterile 2ml vials which were then labelled appropriately and flown to Nairobi immediately for storage in a freezer (-20°C) at the AMREF laboratory. After collection of all samples, they were

transferred to the immunology laboratory at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi for testing.

3.6 Serological Tests

All the 774 livestock and human samples were subjected to Rose Bengal Plate Test (RBPT) and Competitive Enzyme-Linked Immunosorbent Assay (cELISA).

3.6.1 Rose Bengal Plate Test (RBPT)

Rose Bengal (RB) antigen obtained from Veterinary Laboratories, Kabete, serum samples and the test plate were warmed up to room temperature (23°C) and wells in the plate labelled with specimen numbers. Using a micropipette, 30f.il of a labelled sample were placed into the corresponding well in the plate followed by 30^il of well mixed Rose Bengal reagent. The two were then mixed thoroughly with an applicator stick and the plate then rocked on a rotator at lOOrpm for four minutes. Results were read by examining macroscopically for presence or absence of visible agglutination against a source of light immediately after removing from the slide rotator. Agglutination denoted a positive test while lack of it meant a negative result. Positive and negative controls were used to monitor the performance of the procedure and to compare the patterns for better interpretation.

3.6.2 Competitive Enzyme Linked Immunosorbent Assay (cELISA)

Competitive ELISA kit (COMPELISA, Veterinary Laboratories Agency, UK) was used. The kit is standardized for the diagnosis of brucellosis. The reagents were prepared and the tests carried out as per the instructions of the manufacturer. The optical densities (OD) were measured at 450nm in a microplate photometer (Humareader, Model 18500/1, Awareness Technology Inc., Germany). Sera and controls were run in duplicates to compare the two OD readings for every sample.

A positive/negative cut-off was calculated according to the manufacturer's recommendations of 60% of the mean of the optical density (OD) of the four conjugate control wells. Any test sample giving an optical density equal to or below this value was regarded as being positive. Each plate had six wells for positive control and another six wells for the negative control.

In this study, cELISA was used as a confirmatory test and therefore any sera testing positive on this test was regarded as positive. The brucellosis prevalence was calculated based on this test using the formula below.

Prevalence = (Total number testing positive/Total number of samples) x 100%.

3.7 Geographic Mapping of the study sites

Using a Global Positioning System (GPS) hand held receiver (GARMIN® international Inc. 1200 East 151^{sl} street, Olathe, Kansas, USA.), an accurate location for each of the

sites visited was recorded. The geo-reference data was recorded in terms of waypoint, latitude and longitude and saved in the GPS hand held receiver. The readings were obtained by positioning the GPS receiver so as to have a clear view of the sky, away from buildings, trees or any other form of obstruction. The readings were obtained within 3-4 minutes.

3.8 Data Management and Analysis

All the data obtained from the field was recorded in the notebook and later entered into a computer using Microsoft Excel for ease of handling. The data was later transferred to Genstat® Discovery Edition 2.

The two tests, RBPT and cELISA were carried out on the samples and 2x2 tables (Table 3.2) were developed. Kappa test statistic was used to assess the level of agreement between the two tests (Martin *et al.,* 1987).

Table 3.2 Two by two table for the calculation of the kappa test statistic

		EL1SA Test		
		POSITIVE SERA	NEGATIVE SERA	TOTAL
Rose Bengal Hate Test	POSITIVE SERA	**a**	b	**a** + b
	NEGATIVE SERA	**c**	d	**c**+d
	TOTAL	**a** + **c**	b + d	N

Association between the explanatory (independent) variables and outcome or dependent variable (prevalence of brucellosis) was investigated by logistic regression using Genstat Discovery Edition 2.

The relationships between each explanatory variable and the outcome variable was investigated and any variable that was significantly associated at the $P< 0.05$ level was included in the multivariable models and through forward and backward elimination, the most parsimonious models in which all explanatory variables remained significant at the $P< 0.05$ level was generated.

Spatial data was downloaded into *Arc View* Geographic Information System (GIS) computer program and analysed to develop disease risk maps.

The Z-test for independent samples (Remington and Schork, 1985) was used to determine whether the proportions of animals positive for *Brucella* antibodies differed significantly between Oropoi, Mogilla and Kibish/Kakuma regions.

The null hypothesis (H_0) was:

H_0: There is no difference between the prevalences of brucellosis in the three regions.

$(P1= P2 =P3)$.

The alternative hypothesis (H_a) was:

H_a The prevalence of brucellosis in the three regions are different.

P3).

Where P1= prevalence of brucellosis in Oropoi.

P2=prevalence of brucellosis in Mogilla.

P3=Prevalence of brucellosis in Kibish/Kakuma.

The result was interpreted at 0.05 level of significance.

4. RESULTS

4.1 Household characteristics

The household characteristics for the study of brucellosis infection in livestock and in humans are summarized in Tables 4.1 and 4.2 respectively. In this study, a total of 88 and 174 households were sampled with regard to brucellosis in livestock and humans, respectively.

Out of the 88 livestock owners who were interviewed (Table 4.1), 34 (39%) grazed their animals as individual herds or flocks while 54 (61%) practiced communal grazing. All the livestock owners, however, utilized communal watering points.

A total of 60 (68%) livestock owners introduced new stock into their herds or flock while the remaining 28 (32%) did not. The mode of introducing new stock into the herd or flock was as follows: purchase-6, charity-9, dowry-14, local entrustment credit system-3 and the rest (28) could not disclose how they introduced new stock into their herd or flock.

There were only three (3%) people reporting moderate contact with extension staff. The three made their own attempt to visit the extension office in Lodwar.

In the study of brucellosis prevalence in humans, out of the 174 people sampled (Table 4.2), 24 (14%) were within the age group 19 years and below, 92 (53%) were between

the age group of 20 and 42 years while 58 (33%) were between the age group of 43 and 69 years. A total of 130 people (75%) were males and 44 (25%) were females.

Table 4.1: Household Characteristics in the study of brucellosis prevalence in livestock.

Variable	**Response**	**No. of cattle stockowners**	**No. of Goat stockowners**	**Total (%)**
Grazing management	Individual	28	6	34 (39%)
	Communal	12	42	54 (61%)
Introduction of new stock into the herd or flock in the previous one year.	Yes	26	34	60 (68%)
	No	14	14	28 (32%)
People's level of awareness about brucellosis.	Fully aware.	none	1	1 (1%)
	Partially aware.	40	47	87 (99%)
	Not aware Completely	none	none	0 (0%)
Frequency of contact between livestock owners and extension staff in the last one year.	No contact	22	21	43 (49%)
	Rare (less than two Visits).	18	24	42 (48%)
	Moderate (3-4 times)	-	3	3 (3%)

Of all the people interviewed, 99% had partial knowledge about brucellosis. They knew it as a disease derived from meat (*edeke lo akiring*) and raw milk *(edeke lo akile).* The disease in humans is treated using herbs. They knew that the disease had similar signs to malaria. They did not, however, know that the disease could be transmitted through contact with fresh animal tissues using bare hands. Majority of the people had also encountered cases of infertility, abortions and retained placenta in their livestock. Because they could not relate these signs with brucellosis in livestock, they did not take any precautions when handling such cases. The placenta was usually given to dogs or thrown into the bush.

The lifestyle of the respondents was also considered whereby 102 (59%) were pure pastoralists and the remaining 72 (41%) were either settled or semi-settled.

As regards consumption of raw livestock products, out of the 174 people sampled, 102 (71%) respondents consume raw blood and 147 (84%) consume raw milk.

Among the people interviewed, 95% had close association with livestock through sharing of compound with their animals, sharing the house with livestock especially the neonates and sharing of watering points.

Table 4.2: Household Characteristics in the study of brucellosis prevalence in humans.

Variable		**No. of people (n). N=174**	**Percentage**
Age of person giving a blood sample.	< 19 years.	24	14%
	20-42 years.	92	53%
	43-69 years.	58	33%
Sex of respondent	Male	130	75%
	female	44	25%
Peoples' level of awareness about brucellosis.	Completely aware	8	5%
	Partially aware	166	95%
	Not aware	0	0%
Lifestyle of the people.	Pure pastoralists	102	59%
	Settled/semi-settled community	72	41%
Consumption of raw blood.	Yes	123	71%
	No	51	29%
Consumption of raw milk.	Yes	147	84%
	No	27	16%
Close association with livestock.	Yes	165	95%
	No	9	5%

4.2 Prevalence of bovine, caprine and human brucellosis.

Competitive ELISA test results were used to work out the prevalence of brusellosis. In bovines, the highest prevalence was in Kibish/Kakuma region with 18% (22/120) but 0% (0/40) in Oropoi and Mogilla regions. The overall prevalence of brucellosis in bovines in the entire study area was 11% (22/200).

In caprines, the overall prevalence in the study area was 13% (52/400), with 25% (20/80) in Oropoi region, 18.8% (15/80) in Mogilla region and 7% (17/240) in Kibish/Kakuma region.

In humans, the overall prevalence was found to be 17% (30/174) with 30% (8/26) in Oropoi, 23% (9/39) in Mogilla and 12% (13/109) in Kakuma/Kibish region. Vaccination of livestock against brucellosis had never been implemented in Turkana District and therefore the seropositivity was likely due to exposure to the infection (fable 4.3).

The prevalence figures obtained in the three regions were compared. In humans, there appeared to be no significant difference ($p>0.05$) in the prevalence of brucellosis between Oropoi and Mogilla, same for Mogilla and Kibish. However, there appeared to be a significant difference ($p<0.05$) in Oropoi and Kibish. In bovine, there appeared to be a statistical difference ($p<0.05$) between the prevalence of brucellosis in Mogilla and Kibish. It was the same for Oropoi and Kibish. For goats, there appeared to be no

statistical difference ($p>0.05$) between the prevalence of brucellosis in Oropoi and Mogilla. However, there appeared to be a statistical difference (($p<0.05$) between Oropoi and Kibish, and same for Mogilla and Kibish.

When the prevalence for the different species were compared, there appeared to be no statistical difference ($p>0.05$).

The seroprevalence of brucellosis by region and by species is presented on maps in figures 4.3, 4.4, 4.5 and 4.6.

Table 4.3 Brucellosis seropositivity of Bovines, Caprines and Humans using RBPT and cELISA, of blood samples obtained in Northern Turkana District, 2006-2007.

Species	**Region**	**No. of samples.**	**No. positive (%) RBPT**	**No. Positive. (%) cELISA**
Bovine	Oropoi	40	0 (0%)	0 (0%)
	Mogilla	40	0 (0%)	0 (0%)
	Kibish/Kakuma	120	7 (5.8%)	22(18.3%)
	Total Bovines	**200**	7 **(3.5%)**	**22(11%)**
Caprines	Oropoi	80	2 (2.5%)	20 (25%)
	Mogilla	80	6 (7.5%)	15(18.8%)
	Kibish/Kakuma	240	0 (0%)	17(7.1%)
	Total Caprines	**400**	**8 (2%)**	**52 (13.0%)**
Humans	Oropoi	26	1 (3.85%)	8 (30.8%)
	Mogilla	39	0 (0%)	9 (23%)
	Kibish/Kakuma	109	2(1.8%)	13(11.9)
	Total Humans	**174**	**3(1.7%)**	**30(17%)**

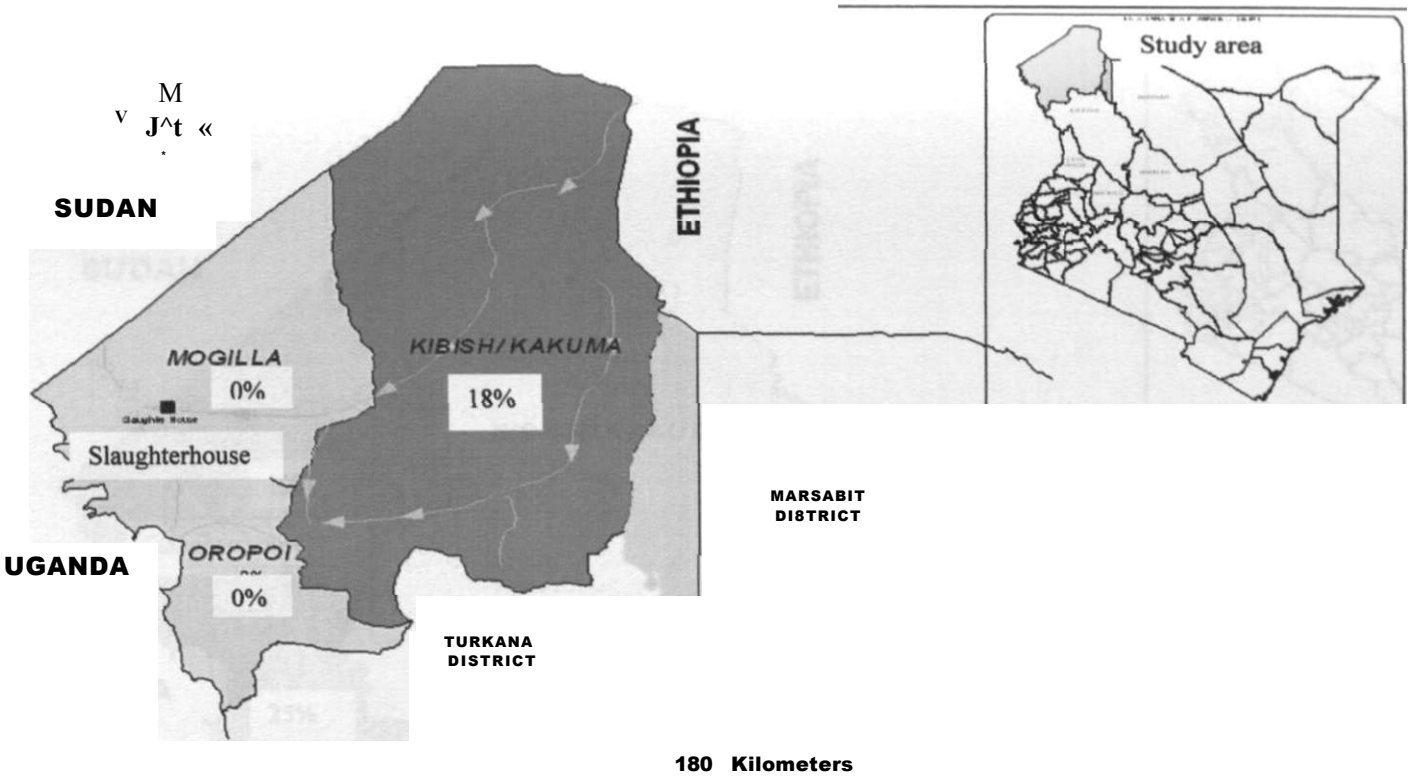


Figure 4.1: A map showing seroprevalence of brucellosis in cattle in northern part Turkana, 2006-2007.

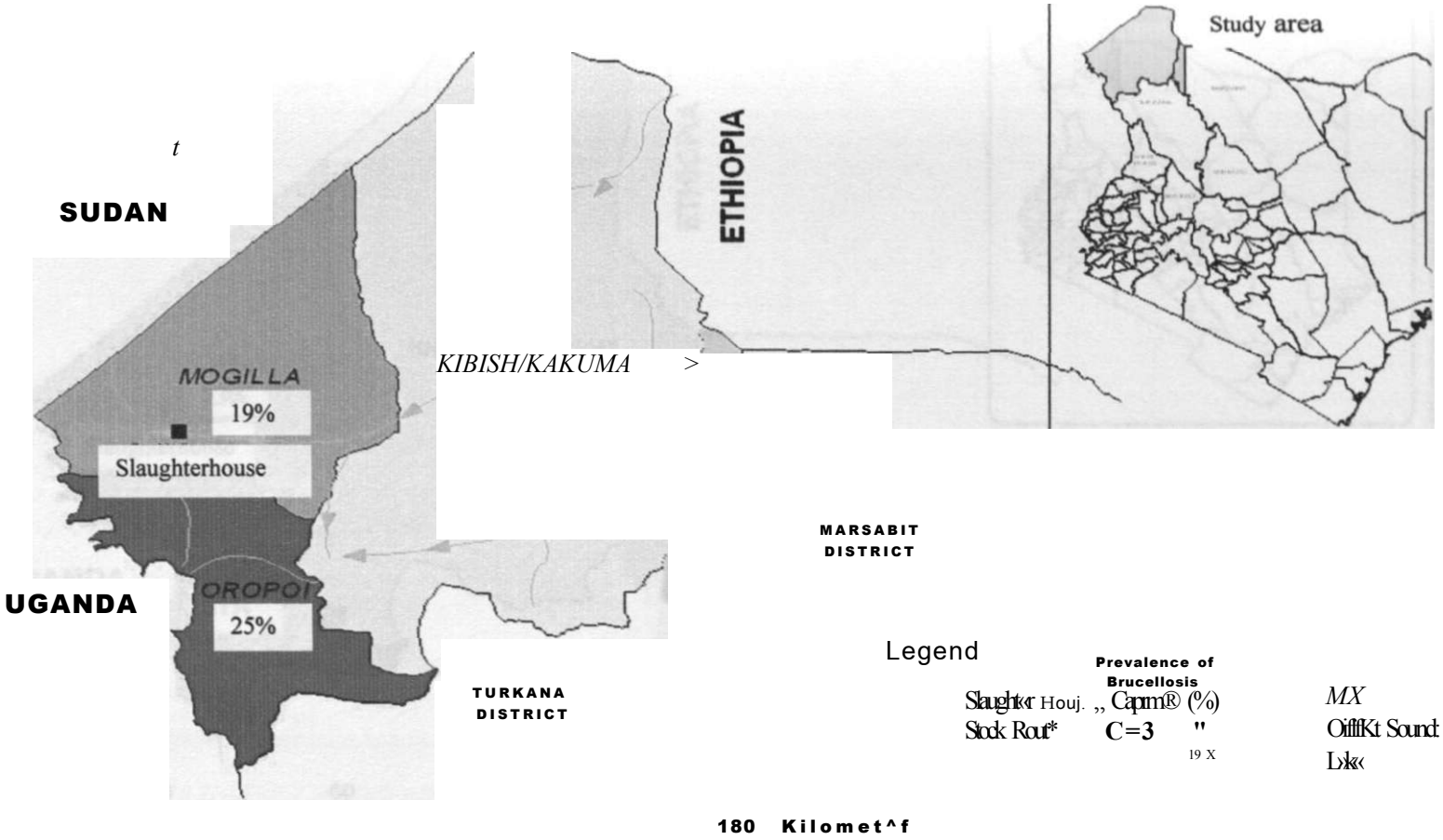


Figure 4.2: A map showing seroprevalence of brucellosis in goats in northern part of Turkana, 2006-2007.

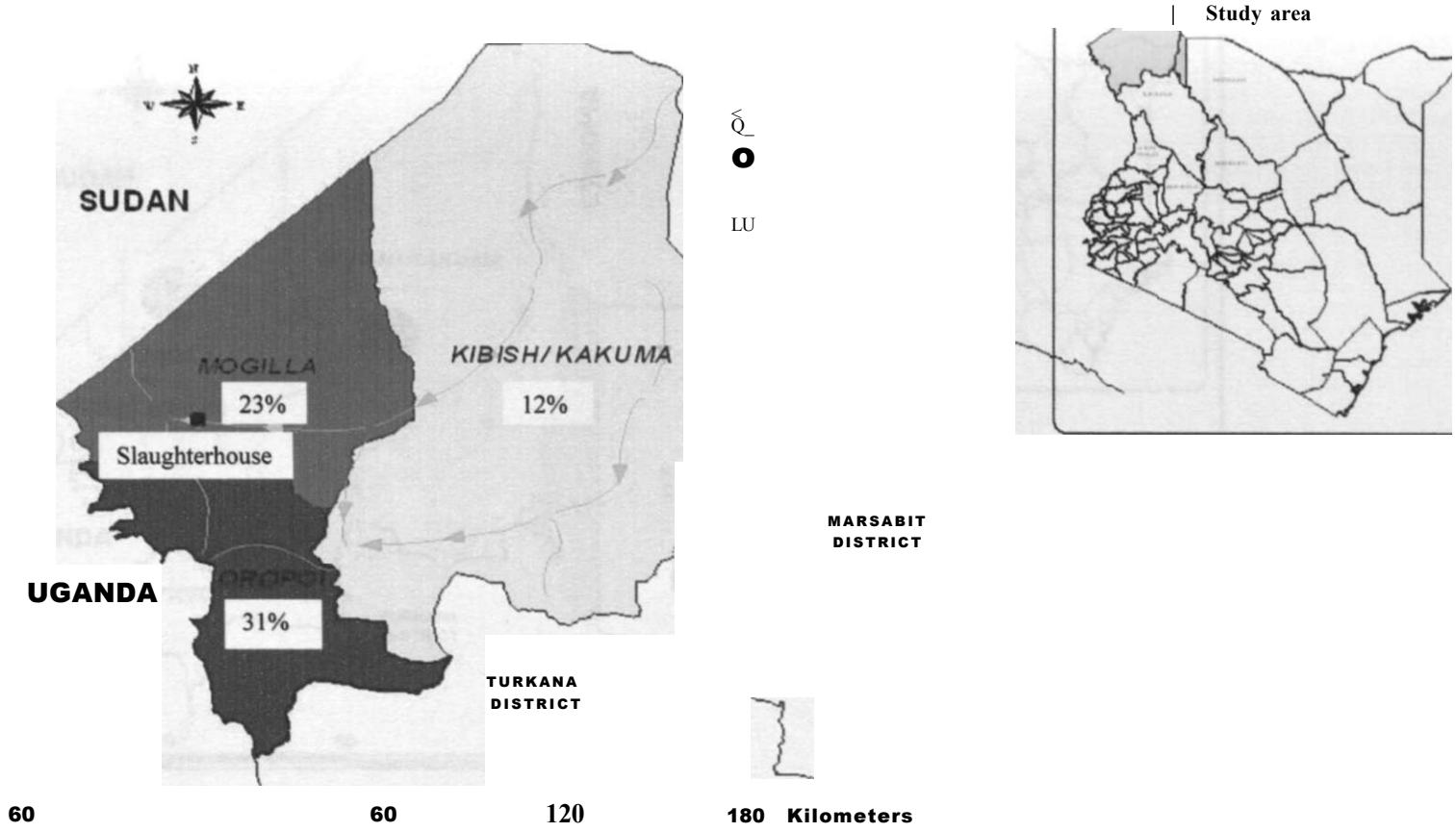


Figure 4.3: A map showing seroprevalence of brucellosis in humans in northern part of Turkana, 2006-2007.

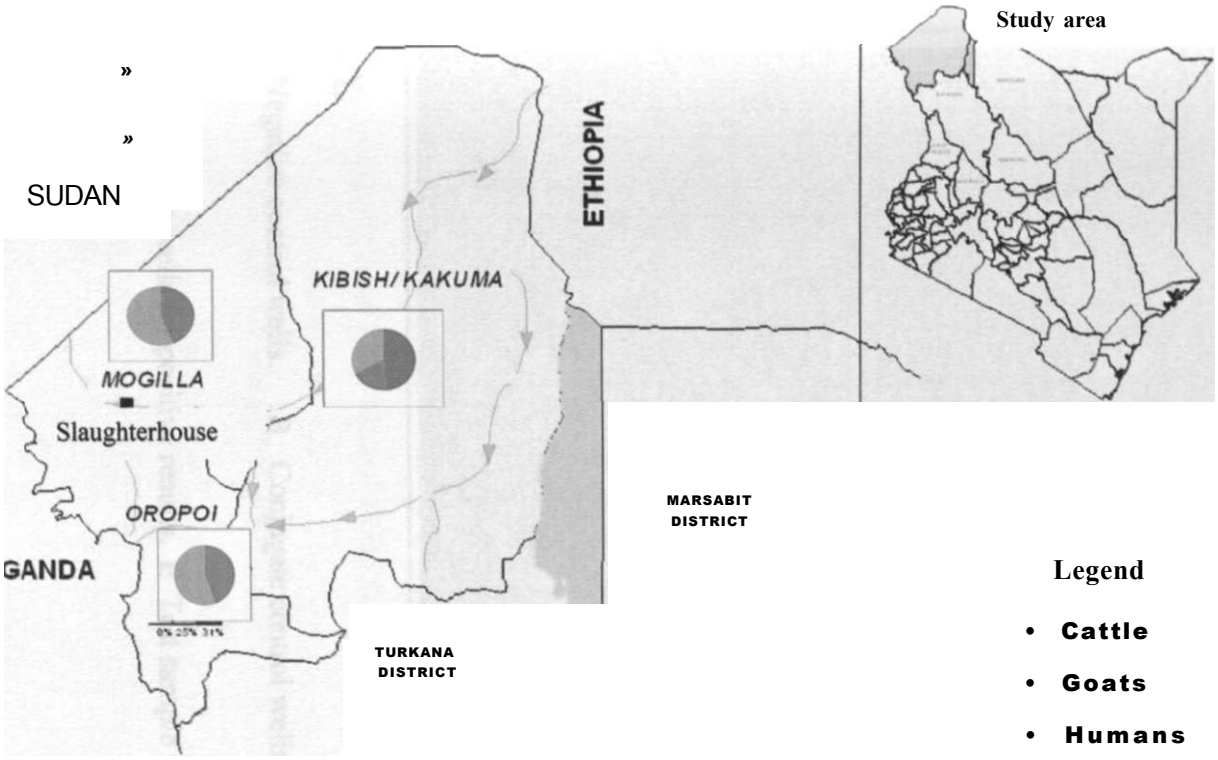
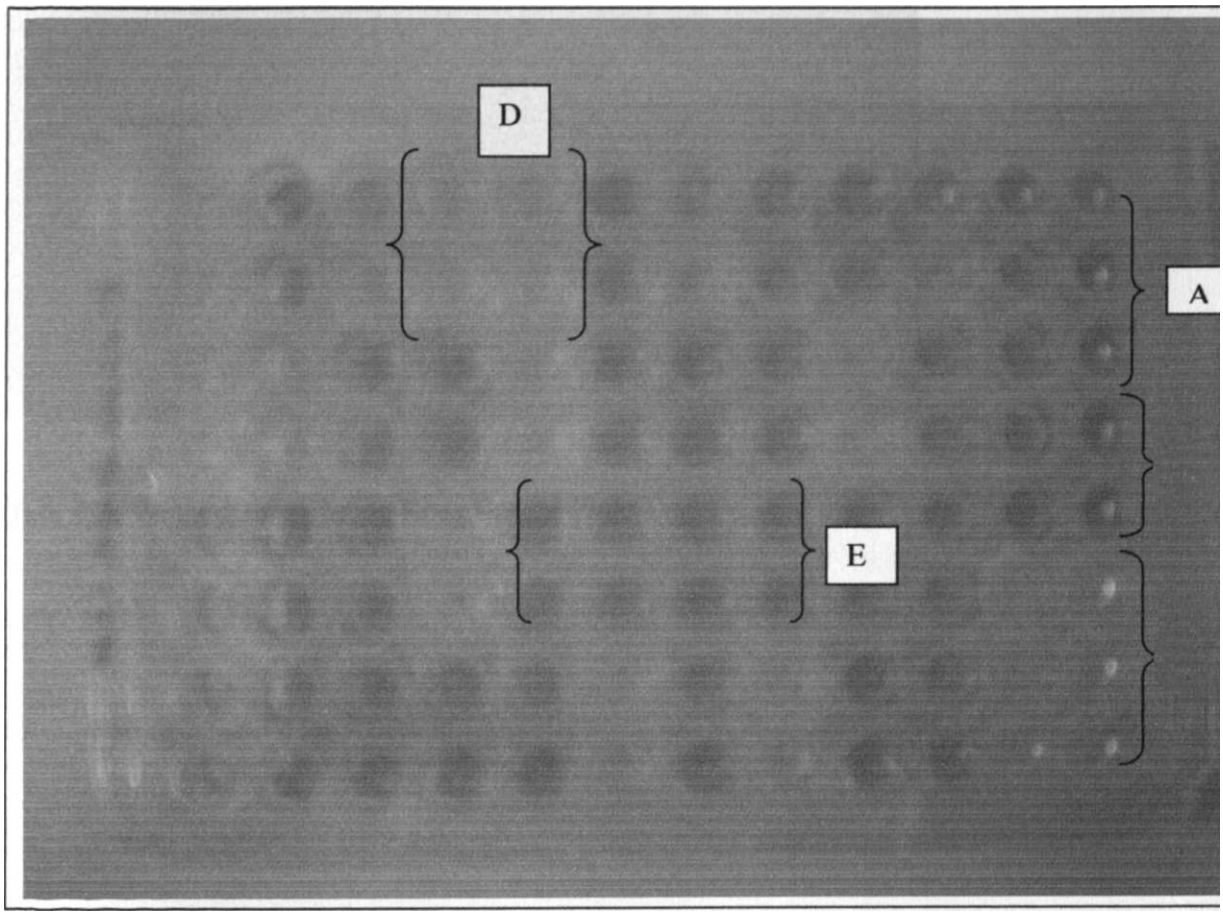


Figure 4.4: A map showing brucellosis seroprevalence in cattle, goats and humans in northern Turkana, 2006-2007.

The positive and negative readings for the two tests cELISA and RBPT used in this study are depicted in plates 4.1 and 4.2 respectively.

Plate 4.1: A plate used to demonstrate competitive ELISA test.



KEY.

A **Negative control wells.** **B Conjugate control wells.** **C Positive control wells.**

D Test sample wells for positive results. E **Test sample wells for negative results.**

Plate 4.2: Wells demonstrating Rose Bengal Plate Test.

KEY.

A: Serum negative for RBP test.

B: Serum positive for RBP test.

4.3 Comparison of Rose Bengal Plate Test and cELISA using the kappa statistic

Tables 4.3, 4.4 and 4.5 show the numbers used to compute the level of agreement between cELISA and Rose Bengal Plate Test in bovines, caprines and humans respectively.

Table 4.4: Comparisons of cELISA and Rose Bengal Plate Test in bovines

		cELISA Test		
		POSITIVE SERA	**NEGATIVE SERA**	**TOTAL**
Rose Bengal Plate Test	**POSITIVE SERA**	**7**	**0**	**7**
	NEGATIVE SERA	**15**	**178**	**193**
	TOTAL	**22**	**178**	**200**

The computed Kappa statistic was a moderate 0.45.

Table 4.5: Comparisons of cELISA and Rose Bengal Plate Test in caprines

		cELISA Test		
		POSITIVE SERA	**NEGATIVE SERA**	**TOTAL**
Rose Bengal Plate Test	**POSITIVE SERA**	**8**	**0**	**8**
	NEGATIVE SERA	**44**	**348**	**392**
	TOTAL	**52**	**348**	**400**

The computed Kappa showed that the two tests agreed slightly (Kappa is 0.24).

Table 4.6: Comparisons of cELISA and Rose Bengal Plate Test in humans

		cELISA Test		
		POSITIVE SERA	NEGATIVE SERA	TOTAL
Rose Bengal Plate Test	POSITIVE SERA	**3**	**0**	**3**
	NEGATIVE SERA	**27**	**144**	**171**
	TOTAL	**30**	**144**	**174**

The computed Kappa showed that the two tests agreed slightly (Kappa is 0.16).

4.4 Trends of human brucellosis as per records at health facilities in Turkana District

Figure 4.1 shows the disease trend as indicated in the laboratory records at Lodwar District Hospital (LDH), Kakuma Mission Hospital (KMH), AMREF clinic (AMREF) and A.I.C. Health centre (A.I.C). The data obtained from these institutions was however limited because of various reasons. The only rccords seen for the year 2004 were for the month of January. There were no records for the year 2005. The inconsistency in records was reported to be due to lack of testing reagents. The patients examined at the hospital came from all parts of the District but majority were from the central part. At Kakuma Mission Hospital, patients were not tested for brucellosis in the months of February and March 2006, also due to lack of testing reagents. However, out of the 534 tests carried

out in the rest of the months of 2006, only 6 were positive. The AMREF clinic was not operational most of the times in the year 2006; nine out of the 37 samples tested in 4 months were positive. The clinic wound up its activities in October, 2006.

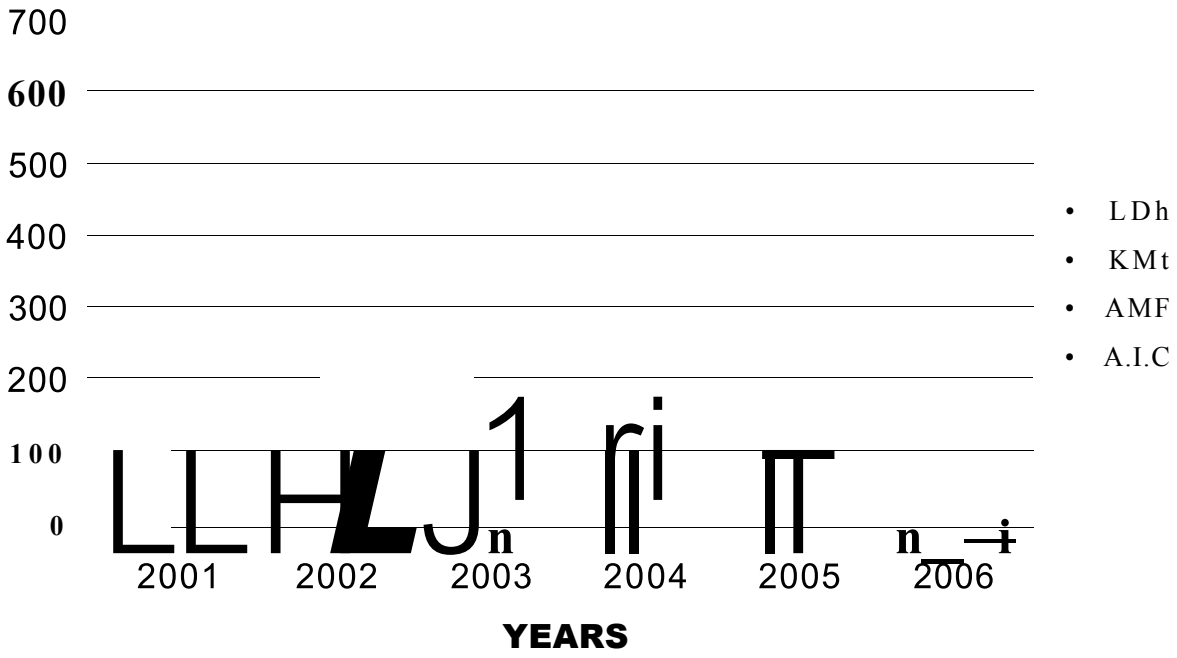


Figure 4.5. Cases of brucellosis in humans in Turkana District as per Laboratory records

KEY

LDH= Lodwar District Hospital. **KMH= Kakuma Mission Hospital.**

AMREF = Amref Health Centre: Lokichoggio. **A.I.C = A.I.C Health Centre, Lokichoggio.**

4.5 Risk factors of brucellosis

4.5.1 Risk factors of brucellosis in univariate analysis

The effect of various risk factors on brucellosis seropositivity in humans in univariate analysis is presented in Table 4.7.

The analysis showed that only two variables were significant at 95% confidence interval. The lifestyle of the people, depending on whether they are pastoralists or settled/semi settled is significant (Odds Ratio of 3.4, p-value of 0.007).

The second significant variable is consumption of raw blood with an Odds Ratio of 3.15 and p-value of 0.025.

The other variables which included age, sex, consumption of raw milk, level of awareness about brucellosis and close association with livestock were not significant at 95% confidence interval.

In livestock, the only significant variable was the grazing system used by the livestock owners (bovines: OR=9.5, Chi square=21.67 at 1 df and $p<0.001$; caprines: OR^O.3, Chi square=8.93 at 1 df and $p=0.003$).

Table 4.7: The effect of various risk factors on brucellosis seropositivity in humans in univariate analysis.

Variable		**No of observations.**	**Positives. Number, (%)**	**Odds Ratio**	**p-value.**
Age	<19 years	24	6 (25%)	1.75	0.278
	20-42 years	92	12 (13)	0.53	0.12
	43-69 years	58	12 (21%)	1.4	0.395
Lifestyle	Pastoralists.	102	24 (23%)		
	Settled/semi Settled group.	72	6 (8%)	3.4	0.007
Sex	Male	130	23 (18%)		
	Female	44	7 (16%)	1	0.785
Consumption of raw **milk.**	Yes	147	28 (19%)		
	No	27	2 (7%)	2.94	0.141
Consumption raw blood	Yes	123	26 (21%)		
	No	51	4 (8%)	3.15	0.025
Awareness level.	Partially	166	30 (11%)		
	Fully aware	8	0 (0%)	oo	0.186
Association with Livestock	Close	165	30 (11%)		0.160
	Non	9	0 (0%)	oo	

4.5.2 Risk factors of brucellosis in multivariate analysis

Tables 4.8, 4.9 and 4.10 show the multivariate analysis of the risk factors of brucellosis in bovines, caprines and humans respectively.

In bovines, the grazing system remained significant (Table 4.8). The same was true for the caprines (Table 4.9).

Table 4.8: Model fitting for risk factors for brucellosis seropositivity in bovines.

Model	Residual df	Residual deviance	Change in df	Change deviance	p-value	comment
constant	199	97.22	.	.		
Constant + grazing	198	78.19	1	19.03	**0.001**	Significant
Constant + grazing+Introd	197	75.65	1	2.54	0.01	Not significant

Key: Introd = Introduction of new stock into the herd,

df =degree of freedom.

Table 4.9: Model fitting for risk factors for brucellosis in caprines.

Model	Residual df	Residual deviance	Change in Df	Change deviance	p-value	comment
constant	399	212.3	-	-	<0.001	
Constant + grazing	398	204.8	1	7.5	0.006	Significant at 95% CI
Constant + grazing+Introd	397	204.7	1	0.1	0.022	Not significant at 95% CI

Grazing= grazing system used by the livestock owner.

Introd=lntroduction of new stock in the herd or flock,

df= degree of freedom

In humans, although the lifestyle of the sampled people was significant in the univariate analysis, it was found not significant when combined with raw blood consumption and is thus a confounder. The lifestyle of the sampled people had an association with brucellosis prevalence (chi-square of 6.83, OR of 3.38) and also had an association with taking raw blood (chi-square of 26 and OR of 7.9 and $p<0.001$) at 95% confidence interval and is therefore considered a confounder. The only significant factor in the model is consumption of raw blood with OR=3.15 (1.67-4.63) at 95% confidence interval.

Table 4.10: Model fitting for risk factors for brucellosis seropositivity in humans.

[Model	Residual df	Residual Deviance	Change in df	Change deviance	p-value	Comment
constant	173	105.6	-	-		
Constant + raw blood	172	101.3	1	4.3	0.038	Significant
Constant + Lifestyle	172	99.4	1	6.2	0.013	Significant
Constant + Lifetyle+ rbld.	171	98.7	1	0.7	0.031	Not significant.
Constant + rbld+ aware	171	99.95	1	1.35	0.059	Not significant
Cons+Lifestyle+rbld + aware	170	97.55	1	1.2	0.045	Not significant
Cons+Lifestyle+rbld +association.	170	97.07	1	1.7	0.036	Not significant

Key

Raw blood=rbld=raw blood consumption. Association=close association with livestock.

Aware= level of awareness about brucellosis. df= degree of freedom.

Lifestyle=Lifestyle of the study population as pastoralists or non-pastoralists (settled or semi settled).

5. DISCUSSION

In this study, the overall prevalence of brucellosis in northern Turkana was 11% in cattle, 13% in goats and 17% in humans by cELISA test, which is more sensitive and specific than RBPT. There was no history of vaccination of livestock against brucellosis in Turkana District. Therefore, the sero-positivity figures obtained were a reliable estimate of animals and humans exposure to *Brucella* infection. The high prevalence of brucellosis in Turkana is consistent with other findings which show that the disease is more prevalent among nomadic pastoralists (Schelling *et al.,* 2003). The Turkana people, like other nomadic pastoralists, keep large herds of cattle and flocks of sheep and goats, which mix freely creating a very conducive environment for transmission of the disease. They also have very close association with their livestock and therefore a high probability of contracting the disease from them.

The prevalence of brucellosis in the study area was found to be higher in goats (13%) than cattle (11%). This is probably due to the grazing system practiced by the Turkanas for the two livestock species whereby cattle are usually grazed ahead of the goats, sheep and camels. This would therefore mean that goats will consume pastures which are contaminated unlike cattle. It is also believed that cattle belong to men while sheep and goats belong to women and children. Whereas cattle are taken to the grazing field by adult men, the goats are taken care of mostly by children. The children may not take the necessary precautions to ensure pastures are not contaminated at the time of kidding. In contrast, the adult men who take care of cattle will most likely remove the aborted fetuses

and placenta from the grazing fields at the time of calving. The Turkana believe that if milking animals consume the placenta, then it will produce little milk. These are some of the possible explanations as to why the prevalence appeared higher in goats than in cattle.

In the Oropoi and Mogilla areas of northern Turkana, the prevalence of brucellosis in goats was high (25% and 19% respectively), but no case was detected in bovines (0%). In humans, 31% and 23% reactors were detected in the two regions, respectively. This was an unusual finding because although *Brucella spp.* tend to discern host predilection in causing overt disease, cross-infection in both domestic and wild animals is not uncommon. The zero brucellosis prevalence in cattle while being high in goats is therefore unexpected. This finding suggests that brucellosis in humans is more likely due to *Br. melitensis*, which is more pathogenic than others and most associated with goats. This finding is consistent with that of Cooper (1992) who showed that the greatest risk for human brucellosis is associated with products derived from sheep and goats as opposed to camels and cattle. Zinsstag *et al.* (2005) has also demonstrated that 90% of human brucellosis was small-ruminant derived. In this study, testing the sheep could have given an indication of the extent of their possible contribution to human infection, but this was not done. However, although sheep were not tested for, they are kept together with goats and B. melitensis infects them with equal measure, which means the base source for human infection was broader. The Turkana community is known to depend on goats and sheep much more than cattle for their daily sustenance while cattle are used more in important functions such as marriage transactions, mortuary rituals and major sacrifices (Barret, 1998).

The high prevalence rate in northern Turkana is expected because of the uncontrolled movement of both livestock and humans at the border points across the country to Sudan, Ethiopia and Uganda. This happens as they graze their livestock especially during the dry season; cross border trade is also common at the time when the Turkana people are at peace with their neighbours and finally through cattle rustling which is a very common practice. In southern Sudan, the prevalence of brucellosis in cattle was 20.2% using Rose Bengal Plate Test (McDermott *et al*., (1987). In Ethiopia, a prevalence of 10% was found in cattle using the Complement Fixation Test (Eshetu *et al*., 2005). A study was carried out in eastern and western Uganda in goats using tube agglutination test and brucellosis card test whereby 2% prevalence was found. These studies show that brucellosis is endemic in the countries which are neighbouring Turkana District.

Slaughter of livestock is carried out indiscriminately among the Turkana community during various ceremonies such as initiation, wedding and others. The slaughter process is done using bare hands. This practice makes the people to be exposed to *Brucella* organisms. With the construction of the abattoir at Lokichoggio and peripheral slaughterhouses which are strategically located in the study area, efforts are being made to have centralized slaughter points. In such a situation, the necessary precautions will be taken to minimize the spread of the disease because hygienic standards will be maintained. The trained meat inspectors will also take the necessary precautions such as the use of protective gear.

The eating habits of the Turkana people contribute to the high prevalence of brucellosis in humans. Eighty four per cent (n=174) of the respondents reportedly consumed raw milk and 71% consumed raw blood. This practice predisposes them to brucellosis. Even for those who cook or roast the meat, they do not do this properly and this may have contributed to the high prevalence figures of the disease.

The level of awareness about brucellosis is probably a factor contributing to the high prevalence of brucellosis in the area under study. Among the respondents interviewed with regard to brucellosis in humans, 5% (n=l74) were fully aware of brucellosis with regard to sources of the infection, transmission and prevention. Among those interviewed with regard to brucellosis in livestock, only 1 % (n=88) knew all aspects of brucellosis infection. The rest of the respondents (99% of respondents for brucellosis in livestock and 95% of respondents for brucellosis in humans) had very limited knowledge about brucellosis. They only knew that brucellosis was transmitted through consumption of meat and milk *(edeke lo airing'* and *edeke lo akile*) and that the disease was treated with local herbs. The people interviewed did not relate brucellosis to abortion and retained placenta in livestock. This makes them handle abortion materials and placenta without any protection or hygienic consideration. All the livestock owners encountered said they had encountered cases of abortion and retained placenta at one time within their flocks or herds. The aborted foetuses and retained placenta were reportedly thrown in the bush or given to dogs. In some instances, the respondents said the aborted fetuses were cooked and given to small children. This, therefore, suggests that brucellosis is endemic in the area but due to lack of awareness and poor hygienic practices employed in handling

aborted fetuses and retained placenta, they contribute to the spread of the disease. Hand-washing is not routinely practiced following contact with infected animals or materials. This is partly due to shortage of water and also because majority of the pastoralists do not associate the disease to abortions and retained placenta.

Among the respondents for brucellosis in humans, 95% (n=174) had very close association with livestock. They shared the compound and watering points with their livestock. They also shared premises with neonates. This poses a high risk to infection. In other instances, animals which kidded or calved while in the field, the neonates were reportedly carried using bare hands and because of the limited knowledge about the disease, no effort was made to wash the hands.

Communal grazing was practiced by 61% (n=88) of the respondents. Livestock from various households were left to roam and mix freely on the same grazing field. This is a risky practice and can result in contamination of pastures when infected animals abort or calve leading to transmission of the disease. Similar findings have been shown by Reviriego *et al*(2000), Ghirotti *et al*., (1991) and Kabagambe *et al*., (2001). Cattle which are grazed communally were nine times more likely to have brucellosis than those grazed individually (OR=9.5). However, from this study, it was observed that goats grazed individually were three times more likely to have brucellosis than those grazed communally (OR=0.3). This is an unusual observation and is probably because the study was carried out at a time when there was an outbreak of peste des petits ruminants (PPR) in goats, a rinderpest like disease of sheep and goats which causes very devastating losses

(Arid Lands Resources Management Project Monthly Drought Bulletin for January, 2007). It is a practice among the Turkana that if there is an outbreak of a disease with devastating effects, the affected herd or flock will be isolated and will not be allowed to mix with the clean ones. The affected herds and flocks will therefore graze individually and will even be watered after the other livestock had been watered. The high number of individuals grazing their flocks and herds individually rather than communally is therefore most likely because there are affected by PPR. The flocks that are affected by PPR were isolated from the rest and thus grazed individually. But because of limited watering points, these animals were still watered at the same watering points with the other flock but only after the clean flocks had been watered.

As regards contact with veterinary extension staff, 49% (n=88) said they had not had any contact with them in the last one year while 48% had had less than two contacts in the same period. This shows that there were few veterinary extension staff in Turkana District and apparently they were not facilitated to meet the pastoralists. The limited contact between veterinary extension staff and livestock owners means that there was nobody to assist in awareness creation about brucellosis.

Studies have demonstrated that laboratory testing is a prerequisite for proper diagnosis of brucellosis in both humans and animals (Smits and Cutler, 2004). Studies have also highlighted the challenges encountered especially in a pastoral setup like Turkana when carrying out laboratory diagnosis (McDermott and Arimi, 2002). In humans, brucellosis presents signs and symptoms similar to other flu-like conditions such as malaria, typhoid,

streptococcal infections and rheumatic fever (Muriuki *et al.,* 1997; Mutanda, 1998; Maichomo *et al.,* 1998; 2000).

Two laboratory tests, Rose Bengal Plate test (RBPT) and Competitive Elisa (cELISA), were used in this study. cELISA is known to be more sensitive than RBPT (OIE, 2000). The RBPT tested fewer samples as positive compared to cELISA and all samples testing positive on RBPT were positive on cELISA. Agglutination tests including RBPT are not recommended for diagnosis of chronic brucellosis since these tests mainly detect IgM which normally declines with time and even becomes undetectable in chronic cases (OIE, 2000). It has also been demonstrated that most laboratory technicians encounter difficulties in conducting the RBPT which is the commonest test in rural health centres (Maichomo *et al.,* 1998). In this study, the problem was not encountered because visible agglutination was checked by the investigator against a light background and confirmed by an experienced technologist.

Cross-reactions between *Brucella spp* and *Yersinia enterocolitica* 0:9 is known to occur. Certain members of Enterobacteriaceae *(Salmonella enteritidis, Salmonella typhimurium* and *Escherichia coli)* also cross react in serological tests (Radostits *et al.,* 2000). The cELISA discriminates false positive results arising from cross reacting anti-LPS antibodies in the above named bacteria (Nielsen et al., 2004; Portanti *et al.,* 2006). It is unlikely that *Y.enterocolitica* 0:9 played a role in this study because the bacteria mainly occur in temperate regions and only induce short term serologic reaction (Shey-Njila *et al.,* 2005).

The agreement levels between RBPT and cELISA were seen to be very low especially in caprine and humans (0.24 and 0.15, respectively). There was a moderate agreement in cattle (0.45). Whereas RBPT is known to be simple, sensitive and specific, studies have pointed out that specificity and sensitivity varies depending on settings and experience of the investigator (Maichomo *et al.,* 1998).

Low seroprevalence was recorded amongst the settled/semi-settled group who stay around towns. This group of people keep relatively few animals, usually the small stock. They are less mobile and have better water supply. A similar observation was made in Northern Jordan (Abo-Shehada *et al.,* 1996). This group of people also have the advantage of having social amenities such as schools, health facilities and churches within their reach and are therefore more informed and enlightened than their counterparts in remote areas regarding hygiene and health in general.

The prevalence of brucellosis in humans was much higher than it was portrayed by the hospital records. This was because testing for brucellosis was not carried out consistently in the hospitals. This was attributed to lack of reagents. It is also possible that many other people do not access the health facilities because of the long distances. The average distance to the nearest health facility in Turkana District is 50km (Turkana District Development Plan, 2002-2008). The other reason is that laboratory tests are carried out at a cost. Each test costs Ksh.60.00 which is considered unaffordable to majority of the pastoralists and therefore they end up not undergoing the test.

The geographic mapping shows that the three strata as defined by the stock routes have different brucellosis prevalence figures. In the Kibish/Kakuma region with the longest stock route, had the highest livestock population with a high proportion being cattle compared to goats (DVO Report, 2006). The region had the highest prevalence figures for cattle but the lowest figures for caprine and human. This was probably due to the big area that is traversed by the cattle and therefore high chances of ingesting contaminated pastures. But with the relatively low goat population, the goat-human interaction is low and therefore the low prevalence figures in humans. The pastoralists in this region are also known to engage more in cross-border movement through cattle rustling. On the other hand, Oropoi and Mogilla are relatively smaller strata and the pastoralists keep relatively less cattle compared to goats. The pastoralists also move over relatively shorter distances. The prevalence figures were zero in cattle and very high for goats and humans probably because of the high goat-human interaction.

The study demonstrated that the prevalence of brucellosis is high in northern Turkana (11% in cattle, 13% in goats and 17% in humans) and therefore presents a big public health problem.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this study, the following conclusions can be made:-

i. The estimated sero-prevalence for brucellosis in northern Turkana is 17% in humans, 13% in goats and 11% in cattle based on Competitive ELISA. The prevalence is high and presents a serious economic and public health problem.

ii. Like all pastoralists the world over, the following factors were observed.

   a. The nomadic lifestyle, communal grazing, unregulated movement of livestock dictated by availability of pastures, water, security situation and disease outbreaks in both livestock and humans.

   b. Rampant and unregulated slaughter of livestock amongst the pastoralists without taking any precautions.

   c. Consumption of raw milk products, raw blood and raw or undercooked meat.

   d. Inadequate information regarding brucellosis amongst the people and especially transmission pathways.

   e. Close association with livestock.

   f. Inadequate health and veterinary services to address the knowledge gaps about brucellosis and put in place the necessary control measures.

g. Poor hygiene practices arising from inadequate water and low awareness level about brucellosis and contact with potentially infected material such as placenta and aborted fetuses.

iii. There was no significant difference between the proportion of cattle, goats and humans having brucellosis.

iv. There is low level of agreement between Rose Bengal Plate test and competitive Enzyme Immunosorbent Assay in all species as shown by the kappa test statistic.

iv. Some health centres test for brucellosis but face shortages of test reagents; records are inconsistent and unreliable.

v. Few people access the health centres and many practice herbal treatment using *'eroronyit'* herbs.

vi. Investigation of brucellosis in livestock is not done because of lack of laboratory services in the District.

vii. The geographic distribution of brucellosis as revealed by the Geographic Information System generated maps shows its pattern in the study area. Brucellosis is not uniformly distributed and this is important in targeting high risk control units for control and guiding research in understanding transmission factors.

6.2 Recommendations

i. The slaughterhouse workers, animal health workers, the pastoralists and all stakeholders need to collaborate and work together to minimize the disease occurrence.

ii. Public health education and publicity campaigns for awareness creation about brucellosis should be carried out. Close liaison between the health and veterinary personnel is critical in the control of the disease accompanied by strong community participation. This should first be directed to the community agents, community animal health workers and the trained herders.

iii. Slaughterhouse workers are most exposed to risk of infection since they are constantly in contact with fresh animal tissues. They should therefore always use protective clothing and gear such as gloves, masks and eye glasses. Also, in addition to the abattoir, attempts should be made to have livestock slaughtered at centralized slaughter points where the necessary preventive measures will be undertaken to reduce infections.

iv. There is need to strengthen laboratory diagnostic capacity through revitalizing veterinary and medical laboratories in the district by training technical staff and providing diagnostic equipments and reagents. This will aid confirmation of cases of brucellosis and thus go a long way in putting in place a surveillance mechanism which forms the basis of any control strategy

This study serves only to provide some baseline information regarding brucellosis in northern Turkana District. It is necessary to carry out an elaborate study for flu-like infections in Turkana and other Districts because there is constant mixing of cattle among the communities living in the region. The study should include camels, sheep and donkeys which are consumed by the Turkana community. The socio-economic impact of the disease should be investigated in addition to identifying the species of bacteria involved. It is important also to investigate other infections in both livestock and humans, which present with clinical manifestations similar to brucellosis or shows cross reactivity on commonly used tests such as Rose Bengal Plate Test.

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8. APPENDICES

8.1 APPENDIX 1. QUESTIONNAIRE FOR DATA COLLECTION ON BRUCELLOSIS IN LIVESTOCK.

GENERAL INFORMATION.

Date: Day / Month / Year:

Name:

Area:_____Location:

Adakar:_____Household No:

GPS:_____-

Region.:

Species:

MANAGEMENT SYSTEM.

Which grazing system is used?

1) Individual herd grazing.

2) Communally free grazing.

3) Other (specify).

Is watering of livestock carried out on individual herds or shared between herds?

1) Individual. 2) Shared. 3) Other (specify)_

C. INTRODUCTION OF NEW STOCK.

viii. Have you introduced new stock on your farm in the last one year?

1) Yes. 2) No.

ix. If yes, how?

1) Cash purchase 2) Charity gift. 3) Dowry.

4)Local entrustment credit agreement. 5) Others (specify):

D. AWARENESS OF THE DISEASE.

x. Have you encountered cases of infertility, abortions or retained placenta in your livestock in the last two years?

1) Yes. 2) No.

xi. How do you handle aborted fetuses?

1) Eat. 2) Throw away in bush. 3) Bury. 4) Give dogs. 5) Other (specify)

xii. How do you dispose the placenta?

1) Throw away in bush. 2) Bury. 3) Give dogs. 4) Other (specify)

xiii Are cases of dystocia assisted?

a. No. b. Yes. c) Do not know,

xiv If yes, is any protection used?

A) No. b) Yes. c) Do not know.

E. CONTACT WITH EXTENSION STAFF.

xv. How frequent did you come in contact with extension officers in the last one year?

1) None. 2) Rare (less than two visits) 3) Moderate (3 - 4 times)

4) Intensive (more than 4 times).

8.2 APPENDIX II. QUESTIONNAIRE FOR DATA COLLECTION ON BRUCELLOSIS IN HUMANS.

A. GENERAL INFORMATION.

i. Date: Day / Month / Year:_____ii. Name:

iii. Division:_____Location:_____iv. Age:

B. CONSUMPTION OF RAW/ UNPROCESSED/UNDERPROCESSED LIVESTOCK PRODUCTS.

v. Do you consume livestock products?

1) Yes 2) No.

vi. Name the livestock products consumed?

1) Milk. 2) Meat. 3) Blood. 4) Others, specify_

vii. Do you process them before consumption?

Milk: 1) No. 2) Yes. 3) Sometimes.

Meat: 1) No. 2) Yes. 3) Sometimes.

Blood. 1) No. 2) Yes. 3) Sometimes,

ix. How are these livestock products processed/ prepared before consumption?

Meat.

Milk

Blood

x. For how long do you keep fermented milk before use?

1) 1 to 3 months. 2) Over 3 months.

C). CLOSE ASSOCIATION WITH LIVESTOCK.

ix. Do you have close contact with livestock?

1) Yes. 2) No.

x. If yes, how?

1) Sharing compound 2) Share house 3) Sharing watering points.

D). AWARENESS OF THE DISEASE.

xi. Have you encountered cases of infertility, abortions and RAB in your livestock?

1) Yes. 2) No.

xii.How do you handle aborted fetuses?

1) Eat. 2) Throw away in bush. 3) Bury. 4) Give dogs. 5) Do not know.

8.3 APPENDIX III. QUESTIONNAIRE FOR THE DISTRICT VETERINARY OFFICER.

1. Have you encountered cases of brucellosis in the District in the last five years?

   1) Yes. 2) No

2. If yes, indicate the number of cases in the table below?

YEAR	CASES	
	CATTLE	SHEEP/GOATS.
2001		
2002		
2003		
2004		
2005		

3. What were the clinical signs observed?

   1) Infertility. 2) Storm abortions. 3) Retained placenta.

   4) Arthritis; swelling of the knee joint (Hygromas). 5) Others (Specify).

4. How was the diagnosis carried out?

   1) Clinical (tentative). 2) Laboratory diagnosis. 3) Other (specify).

How were the cases managed/ controlled?

   2) Treatment. 2) Vaccination. 3) Other (specify).

5. Are there other conditions with similar manifestations in the district?

1) Yes. 2) No.

6. If yes, when did they occur and what was the diagnosis?

When

Diagnosis

7. Do you have adequate extension staff to serve the pastoralists.

1) Yes. 2. No.

8.4 APPENDIX IV. QUESTIONNAIRE FOR THE DISTRICT MEDICAL OFFICER OF HEALTH.

1. Have you encountered cases of brucellosis in the District?

   1) Yes. 2) No.

2. If yes, what are the clinical signs exhibited?

   1) Fever.

   2) Headache.

   3) Joint and body pain.

   4) General weakness.

   5) Sweating.

   6) Chills.

   7) Other, (specify).

3. How is the diagnosis carried out?

   1) Tentative. 2) Laboratory diagnosis. 3) Other (specify).

4. If laboratory diagnosis, which test?

   1) Serological test. 2) Blood for brucella culture.

5. Which serological test is used?

   1) Rose Bengal Plate Test. 2) Serum agglutination test. 3) ELISA. 4) Other (specify).

6. What are the differentials for brucellosis?

   i) 2)

3) **4)**

7. How is the disease managed?

8. Show the general trend of the disease over the last five years in the District.

YEAR	CASES
2001	
2002	
2003	
2004	
2005	