

**» A COMPARISON OF SENSITIVITY OF TEST KITS  
USED FOR DIAGNOSIS OF HUMAN BRUCELLOSIS IN  
KIBERA, NAIROBI, KENYA »**

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This report is my original work and has not been presented for a degree in any other university

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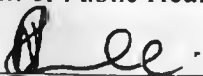
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## **DEDICATION**

To my husband Jack, my sons Jerry and Jamie and my daughter Patricia; for the great love, care and motivation you have given me all along.



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## ABSTRACT

Animal and human health is inextricably linked. People depend on animals for nutrition, socio-economic development and companionship. Yet animals can transmit many different diseases to humans.

Brucellosis is a common zoonosis in Kenya. In areas where the disease occurs, malaria, and typhoid which manifest with similar clinical symptoms also occur. Therefore clinical diagnosis of brucellosis needs confirmation. This requires a test with high specificity and sensitivity. This study was therefore carried out with the objectives of

Increasing the precision in the diagnosis of human brucellosis.

Specific objectives were:

- 1) Determining the sero-prevalence of human brucellosis among patients with clinical signs consistent with brucellosis attending health clinic / hospital in kibera.
- 2) To determine the prevalence of Typhoid and Malaria as differential diagnoses for brucellosis in the above patients.
- 3) To compare the sensitivity of rapid kits used in the diagnosis of human brucellosis in Kenya with Rose Bengal Plate Test and Competitive Elisa.
- 4) To compare Agreement between Rose Bengal Plate test and Competitive Elisa

In Kenya, diseases with flu-like symptoms such as brucellosis, malaria typhoid and paratyphoid pose a diagnostic problem to clinicians as the diseases can occur concurrently in the same patient or in the same locality. A study was carried out by the

University of Nairobi in conjunction with the African Medical and Research Foundation (AMREF) at the AMREF Kibera Community Clinic and Mbagathi District Hospital to determine the occurrence of brucellosis in patients presenting with febrile conditions in a slum setting in Kibera, Kenya.

Patients presenting with clinical symptoms and signs consistent with brucellosis were selected for the study. Blood samples were collected aseptically. A portion was used to make blood films for examining for malaria parasites and blood culturing for *Salmonella* spp. From the other portion of blood serum was extracted and used for diagnosis of brucellosis using competitive Elisa test, Rose Bengal plate test and four other serological test kits from Biotec laboratories, Fortress Diagnostics limited, Tulip diagnostics and Eurocell diagnostics which are used in local hospitals for the diagnosis of brucellosis.

A total of 105 Patients were recruited into the study. Samples collected were tested for brucellosis using on-site rapid test kits at the health facilities. One sample was positive on the Rose Bengal test and five samples were positive for brucellosis on all the other agglutination tests. Two blood cultures were positive for *Salmonella typhi* and one blood sample was positive for malaria parasites. The agglutination kits showed different positive results depending on the Kit used. No sample was positive for brucellosis using Competitive Elisa Test.

The results from this study indicate no prevalence of brucellosis in patients in the population studied based on ELISA. However, the agglutination tests to detect *Brucella* antibodies did not agree with ELISA test which was used as the gold standard for testing brucellosis in this study, thus giving false positive results, and therefore there is a need to re-evaluate the diagnostic tests for brucellosis before their use in health facilities.

# CHAPTER ONE

## 1.0 INTRODUCTION

Brucellosis is considered by Food and Agricultural Organization (FAO), World Health Organization (WHO) and International Organization on Epizootics (OIE) as the most widespread zoonoses in the world. (Anonymous 2007).

*Brucella melitensis*, the cause of caprine brucellosis is the most important cause of human brucellosis. Brucellosis in humans is characterised by fever, chills, weakness, joint pains, night sweats and weight loss and can be confused with malaria, influenza, typhoid fever, Rift valley fever (RVF), rheumatism, leptospirosis, Q-Fever (Coxiellosis) and Psittacosis (Muriuki, 1994)

A previous retrospective study on community health risks in relation to zoonoses conducted in human health facilities that serve Kibera namely Mbagathi District Hospital, African Medical Research Foundation (AMREF) Kibera Community Clinic and Langata Health Clinic revealed possible existence of brucellosis among patients (Njenga et al., 2005). Brucellosis is a zoonosis of both public health and economic significance in most developing countries. In many developed countries, the animal disease has been brought under control, which has led to a subsequent decrease in the number of human cases. The occurrence of the disease in humans is largely dependent on the animal reservoir. Where brucellosis exists in sheep and goats, it causes the greatest incidence of infection in humans (WHO, 2004).

Brucellosis is a true zoonosis in that virtually all-human infections are acquired from animals and human-to-human infection does not play a significant role in transmission. Human infections arise through direct contact with infected animals, including handling

of infected carcasses; indirectly from a contaminated environment; or through consumption of infected dairy products. The disease is therefore an occupational hazard and groups at risk include herdsmen, butchers, veterinarians, laboratory technicians and workers with dairy products (Corbel, 2002).

Brucellosis in humans and animals is increasing in certain parts of the world, especially in developing areas of the Mediterranean Region, Middle East, Western Asia and parts of Africa and Latin America. *Brucella melitensis* especially, being very pathogenic for human beings, constitutes a public health priority. Its recent emergence as a bovine pathogen in intensive dairy farms causes particular concern (WHO, 2004).

Brucellosis in Kenya has been documented in both animals and human beings (Cox, 1966; Oomen, 1976; Kagunya, 1977; Paling et al, 1988; Muriuki, 1994; Maichomo, 1997).

Due to non-specificity of the clinical manifestation of the disease, the diagnosis of brucellosis may be difficult in the early stages when it has to be differentiated from other causes of prolonged fever such as typhoid and malaria. This is especially important considering malaria is the first line of treatment for cases of fever in all the health facilities and only after patients fail to respond to malaria treatment is further investigations done or referrals given. The incidence, prevalence and risk factors for brucellosis in Kenya have been scarcely studied. The few studies carried out are based on selected case follow-ups rather than population-based samples. They, however, indicate that the disease is widespread.

## 1.1 Justification

Increased economic hardships have led to increased rural urban migration. Availability of home and hotel food waste and availability of markets for livestock and their products have led to the increased livestock keeping among the urban poor. Livestock is a means of income generation, household food supply and livelihood security. Middle-income families practise urban dairying to supplement household incomes, while the urban poor engage in the practice as a response to limited alternative livelihood options and food insecurity (Bangura and Gibbon, 1992). However, the effective and efficient utilization of this farming activity needs to address issues of provision of product safety, environmental degradation and the risk of zoonoses (Richards and Godfrey, 2003). Having shown the potential for brucellosis in the Kibera community (Njenga et al 2005), the magnitude of the disease is difficult to establish without conducting further research, as many residents visit their rural homes regularly and thus could be infected from there. However the observed animal husbandry practices of animals roaming the streets and scavenging for feed in open spaces, poor sanitation, low and incorrect knowledge on zoonoses could all contribute to transmission of zoonoses. The sale of curdled milk as “*maziwa lala*”, which was observed among milk hawkers, can be a source of brucellosis for Kibera residents because the *maziwa lala* is made from unpasteurized milk, which has not been sold during the day and is at risk of being spoilt incurring losses to milk traders (Omore *et al*, 2005, Kang’ethe et al. 2007).

The purpose of this study was to determine the prevalence of Brucellosis among patients visiting health facilities in Kibera through screening with Rose Bengal, Competitive Elisa and routinely used agglutination tests. Since malaria and typhoid clinically present

similarly and are endemic in Kenya, the patients were also screened for malaria and typhoid using blood slide smear and bacterial culture respectively. Lack of specificity in the clinical features of typhoid fever was noted as early as 1863 by Marston (quoted by Huckstep, 1962), when he added brucellosis (Malta fever) as an important differential diagnosis for typhoid fever. Similar findings were also noted by Endeshaw and Habte (1987) when they suggested that typhoid fever is a great mimic and should be considered in differential diagnosis of patients presenting with fever and bleeding disorders.

## **1.2 Objectives**

### **1.2.1 General objective**

To increase the precision in the diagnosis of human brucellosis.

### **1.2.2 Specific objectives**

1. To determine the sero-prevalence of Human brucellosis among patients attending health facilities in Kibera.
2. To determine the prevalence of Typhoid and Malaria as differential diagnoses for brucellosis in the above patients.
3. To compare the sensitivity of rapid kits used in the diagnosis of human brucellosis in Kenya with Rose Bengal Plate Test and Competitive Elisa.
4. To compare Agreement between Rose Bengal Plate test and Competitive Elisa

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 BRUCELLOSIS

##### 2.1.1 Definition

Brucellosis is a direct bacterial zoonosis (infectious disease common to both humans and animals). In animals it is characterized by abortion, retained after-birth, orchitis, epididymitis and hygromas, impaired fertility, drop in milk yield, which can lead to premature culling (Radostits *et al*, 2000). In humans, brucellosis manifests as fever, chills, sweating, joint pains, malaise and weight loss (OIE/CFSPH, 2004).

##### 2.1.2 Etiology

Brucellosis is caused by bacteria of a genus *Brucella*, which appear as *cocci*, *coccobacilli* and short bacilli, 0.5-0.7 $\mu$ m wide by 0.6-1.5 $\mu$ m long. They are gram negative, non-motile, non-capsulated and non-spore forming rods usually occurring singly but occasionally forming small groups. In culture, with the exceptions of *B. canis* and *B. ovis* strains, which are permanently rough, the colonies are normally smooth, moist, convex, transparent and glistening. However, the organisms can mutate in liquid media, forming rough colonies on subculture (Corbel, 2002). They have a closely related antigenic structure, which makes their serologic differentiation difficult. They are protected from host defences by localizing and proliferating within the cytoplasm of monocytes and reticulo-endothelial cells, and are shed continuously especially by female animals (Jubb



*et al*, 1985).

There are six recognized species of *Brucella* genus; *Brucella abortus*, *B. melintesis*, *B. suis*, *B. canis* that are zoonotic while *B. ovis*, *B. neotomae* are not zoonotic (Radostits *et al*, 2000). The primary hosts are cattle, goats, swine, dogs, sheep and the desert woodland rat (*Neotoma lepida*). Two new brucella species, provisionally called *Brucella pinnipediae* and *B. cetaceae*, have been isolated from marine hosts within the past few years (Pappas *et al*, 2005).

Inter-species transmission of *Brucella* organisms occurs readily. *B. abortus* has been reported to infect cattle, sheep and goats, horses, dogs, pigs and wildlife species like bison, elk, deer, coyotes, wild opossums and raccoons, moose, and the African buffalo (*Syncerus caffer*) and various African antelope species (Radostits *et al*, 2000; OIE, 2004). *B. bacterianus*, has also been reported in the one-humped camel (*Camelus dromedarius*) and in the two-humped camel related to contact with large and small ruminants infected with *B. abortus* or *B. melitensis* (OIE, 2004).

*B. Melitensis* infects sheep and goats, cattle, camel, buffaloes, swine, poultry and humans (FAO/WHO, 2004). *B. suis* infects swine, cattle, sheep and goats, humans, dogs and probably other species (OIE, 2004). *B. canis* infects dogs, foxes, monkeys and man. *B. ovis* and *B. neotomae* do not seem to infect other species besides sheep and desert wood rat respectively.

*Brucellae* organisms have been isolated from animals since 1887 but it was not until 1914 that the first *Brucella* of human origin were identified by Bruce (Carter and Chengappa, 1991).

A number of *Brucella* species have been isolated in Kenya since 1968. *B. ovis* was isolated from rams in Rift Valley Province (Cameron *et al*, 1971). In 1972 all the three biotypes of *B. melitensis* were isolated in sheep and goats (Philpot and Auko, 1972). The organism has also been isolated from human patients (Oomen, 1976). *B. abortus* biotypes 1, 3 and 9 have been isolated from Kenyan cattle (Waghela, 1976). *B. suis* has been isolated from rodents (Heisch *et al*, 1963). No isolation from wildlife has been achieved although serologic evidence of infection has been adduced (Waghela, 1976; Paling *et al*, 1988). Isolation of *B. melitensis* and *B. abortus* from humans has been documented (Oomen, 1976; Maichomo *et al*, 2000).

## **2.1.3 Epidemiology**

### **2.1.3.1 Occurrence of Brucellosis**

Brucellosis is an animal disease of economic importance and a major zoonosis in most countries of the world. The prevalence of infection varies considerably between herds, areas, and countries. Many countries have made considerable progress with their eradication programs and some have eradicated the disease. However, in other countries brucellosis is still a serious disease problem facing the veterinary and medical professional (Radostits *et al*, 2000, Pappas *et al*. 2005).

Brucellosis in cattle is prevalent in the whole of Africa. In the field, *B. abortus* is the main causal organism, although *B. melitensis* infections have been reported and *B. suis* infections suspected (Chukwu, 1985). The prevalence and incidence of Bovine brucellosis is highly variable, usually greater in systems in which large numbers of cattle mix and lowest for small confined herds (McDermott and Arimi, 2002).

*B. abortus* is probably the most widespread due to the universal distribution of cattle, but *B. melitensis* has been the most frequently isolated in cases of human illness. *B. melitensis* as a cause of illness is common wherever goats are raised, but it is especially prevalent in the developing countries (OIE, 2004). The same applies to *B. abortus* and *B. suis* in cattle and pig raising areas respectively. *B. melitensis* may have originated from the Middle East where small ruminants were domesticated, but now occurs widely throughout the world (Alton, 1987).

The development of large-scale livestock production (for example, sheep in the Middle East) has led to serious epidemics, sometimes in areas where the disease has not been common. Brucellosis is not new in Africa (Chukwu, 1985). In Kenya, animal and human brucellosis have been reported practically in every district surveyed. Cox (1966) observed many cases in the North where he performed serological tests under rural conditions. Heisch *et al*, (1963) isolated *B. suis* from rodents at the Coast, while Waghela and Gathuma (1975) reported evidence of porcine brucellosis in Kenya.

Clinical cases of human brucellosis have also been described from Machakos District and North Eastern (N.E) Province by Kagunya (1977), where the presence of anti- *Brucella abortus* agglutinins in cattle and camels and those against *B. melitensis* in sheep and goats were reported. Wildlife species have similarly been reported to have anti- *Brucella* agglutinins (Paling *et al*, 1988) although no isolation has been made in Kenya so far. The prevalence of Brucellosis in animals and man is higher in the pastoral areas of Kenya where large numbers of livestock are kept in close contact with the people (Oomen and Wegener, 1982). The very low prevalence in zero grazing reflects very low cattle-to-cattle contacts, even in the absence of specific control measures (Kadohira *et al* 1997;

McDermott and Arimi, 2002). Kagumba and Nandokha (1978) have reported higher prevalence in cattle from Maasai-land and semi-arid areas of East Africa.

### 2.1.3.2 Sensitivity and survival

Within the host, *Brucellae* are protected from the host defense by localizing and proliferating within the cytoplasm of monocytes and reticulo-endothelial cells (Jubb *et al*, 1985). They may remain in the host for life. The ability to survive intracellularly, as well as in granuloma and abscesses, probably accounts for the chronic and relapsing nature of the febrile disease (Baron *et al*, 1994).

The survival of the organism in the environment may play a role in the epidemiology of the disease. Temperature, humidity, and pH influence the organism's ability to survive in the environment. *Brucellae* are very sensitive to direct sunlight. They are moderately sensitive to acid, so that they tend to die out in sour milk and in cheese that has undergone lactic acid fermentation. In dairy processing, *Brucellae* tend to concentrate in the cream fraction of the products thereby making them a common source of human infection (FAO/WHO, 2004). There are reports of *Brucellae* isolation from fermented milk and cheddar cheese; this means that transmission from these dairy products is possible (Minja *et al*, 1998).

The organism can survive in soil, manure and dust for weeks or months, and remain viable in dead fetal material for even longer (Corbel, 2002). *Brucellae* survive well at temperatures below freezing point, at 4 to 8°C in moist environment and in the absence of direct sunlight. They however are readily killed by freeze-thaw conditions, heat, phenol, formaldehyde, quaternary ammonium compounds and pasteurisation at 62.7°C for 30minutes or 71.6°C for 15 seconds (FAO, 2004). In dry conditions they survive only if

embedded in protein.

### **2.1.3.3 Source of infection**

Domestic animals are the main source of contamination of the environment. *Brucellae* are facultative intracellular pathogens with a predilection for the reticulo-endothelial system (RES) and reproductive organs. Involvement of the urogenital system of infected hosts is of great epidemiologic importance. It is the principle route of escape for the organism leading to environmental contamination that is essential for its spread. *B. abortus* achieves its greatest concentration in the contents of the pregnant uterus, the fetus and the fetal membranes, and these must be considered as major sources of infection (Radostits *et al*, 2000). This is because organisms tend to concentrate in the uterus due to high levels of erythritol in the gravid uterus (Jones and Hunt, 1983).

Based on experimental cases, there is evidence of decreasing numbers of organisms when uterine discharges are cultured at sequential parturitions, and that a substantial number of uterine samples from infected cows are culture negative at the second and third parturition following challenge (Radostits *et al*, 2000). The uterus should ideally be negative for bacteria after involution, but some animals may shed the organisms for weeks or even months. Animals shed the organisms and contaminate the environment when the discharges fall in water supplies and feedstuffs or when they come in contact with aborted foetus and infected new calves and this can lead to infection through oral, respiratory, or percutaneous routes.

The second major route of escape for *Brucellae* is the mammary gland. Excretion of the organism in the milk is usually intermittent but appears to be more common during late

lactation and can continue for several years. In cattle vaccinated before infection, the degree of excretion of *B. abortus* in the milk is less than in non-vaccinated animals (Radostits *et al*, 2000). The milk looks normal but it has an increased somatic cell count (Jubb *et al*, 1985), and a chronic suppurative inflammation of the mammary gland is observed on microscopic examination. Other modes of environmental contamination are through infected carcasses, aborted fetuses and fetal membranes, and the faeces of some infected animals. The pregnant sow is not especially susceptible and the infection is spread by the boar in the semen. Infection of man is mainly by handling pig meat.

#### **2.1.4 Brucellosis in animals**

##### **2.1.4.1 Main reservoir species**

*Brucellae* have both true (primary) and alternative (secondary) animal hosts. Cattle are the true host species for *B. abortus* but infection in other Bovidae is reported like the domestic buffalo in Asia and Middle East, the African buffalo (*Syncerus caffer*), the North American bison and the Yak, which is highly susceptible. Others are waterbuck, impalas, gazelles and Wildebeast (OIE, 2004). Camels, horses, donkeys, swine and sheep have also been incriminated (Radostits *et al*, 2000). Dogs with naturally acquired *B. abortus* infections play an important role in the epidemiology of cattle brucellosis. The relationship between infected dogs and outbreaks of brucellosis in cattle has not only been reported but has also been demonstrated (Forbes, 1990).

*B. melitensis* natural host is the goat but sheep is less susceptible with susceptibility varying among the breed of sheep. The organism is capable of causing disease in cattle and has been isolated from swine. *B. suis* is pathogenic only for pigs and humans although other species, including cattle and horses may be infected, especially if they

share a range with feral pigs (Radostits *et al*, 2000). In most cases it is not easy to differentiate between the various species of *Brucella* because most of the available host range information is derived from serological surveys. The organisms are very close antigenically.

*B. ovis* affects sheep but not goats. This organism can cause epididymitis, orchitis and impaired fertility in rams. Initially, only poor quality semen may be seen; later, lesions may be palpable in the epididymis and scrotum. Epididymitis may be unilateral or, occasionally, bilateral. The testes may atrophy. Some rams shed *B. ovis* for long periods without clinically apparent lesions. Abortions, placentitis and perinatal mortality can be seen in ewes but are uncommon. Systemic signs are rare. (Merks manual 2007)

#### **2.1.4.2 Modes of transmission of animal brucellosis**

Exposure occurs by licking or muzzling of newborns on external genitalia of infected animals, ingestion of feed and water contaminated with secretions, excretions, or tissues especially when aborted animals shed the organisms on vegetation and water sources. Contact infection through skin and mucous membranes may occur from heavily contaminated bedding, while aerosols and droplets generated by tail switching and during parturition facilitate infection through airways and conjunctiva (FAO/WHO, 2004).

Intra-mammary exposure through the teat canal can occur during hand milking due to cross contamination. Infected females may transmit to the conceptus in-utero or through milk post- nately (Radostits *et al*, 2000). Although the organisms localize in the male and female genital tracts, venereal transmission is thought to be insignificant probably because the number of *brucellae* in semen is much lower than that required for infection per-vaginum. However, females can easily be infected during artificial insemination with

semen from infected male animals when semen is introduced directly into the uterus (Noakes *et al.*, 2001). *B. ovis* infection in sheep is thought to be transmitted venereally, from a ram with epididymitis shedding large numbers of organisms in the semen (Carlton and McGavin, 1995). Licking of infected rams' penis and homosexuality is also a common route of infection (OIE manual 2008).

Embryo transfer from infected donors may be achieved without transfer of infection and super-ovulation is unlikely to reactivate the release of *Brucella* into the uterus when embryos are normally collected. Thus embryo transfer should be considered a safe procedure for salvaging genetic material from infected animals (Radostits *et al.*, 2000).

Transmission by ticks and biting insects has been demonstrated experimentally, but their role in natural transmission has not been documented. The organism is taken into the alimentary tract of the housefly but is rapidly eliminated and there is no evidence for a role in natural transmission (Radostits *et al.*, 2000). Other modes of transmission such as waterways, air currents, contaminated equipment and scavengers are remote possibilities when other modes of transmission are eliminated (Ray, 1979; FAO/WHO, 2004).

#### **2.1.4.3. Pathogenesis and clinical disease**

The onset of *Brucella* infection depends on exposure dose, virulence of the organism and natural resistance of the animal to *Brucella*. Resistance to infection is based on the hosts' ability to prevent the establishment of a mucosal infection by the destruction of the invading organism.

Following infection of the host, *brucellae* are subjected to non-specific host defence mechanisms in which they are engulfed by leukocytes. In the leukocytes they multiply within the cytoplasm leading to the eventual rupture and death of the leukocyte. The



*Brucellae* are then released into the host system. Their ingestion by monocytes then takes place leading to the transportation to the regional lymph nodes where an immune response is mounted, which may lead to granuloma formation (Jubb *et al*, 1985). This leads to bacteraemia and generalized infection from 14 days to several months. Localization of the organisms may occur in organs, especially those of reticulo-endothelial system: liver, spleen, bone marrow and lymph nodes, as well as mammary glands, testes and uterus where the organisms may persist for years.

**Cattle:** The predominant sign in pregnant females is abortion or premature or full term dead or weak calves and retained placentas. In the adult non-pregnant cow, localization occurs in the udder, and the uterus if it becomes gravid, is infected from periodic bacteraemia phases originating in the udder. Infected udders are clinically normal but are a good source of infection for calves or humans drinking the milk.

In bulls *Brucellae* may become localized in the testis and other genital organs (epididymis, seminal vesicle and ampullae). Orchitis and epididymitis occur occasionally. One or both scrotal sacs may be affected with acute painful swelling to twice the normal size. The seminal vesicles may be affected and their enlargement can be detected on rectal palpation (Jones & Hunt, 1983).

*B. abortus* can often be isolated from lesions of non-suppurative synovitis in cattle. Hygromatous swellings especially on the knees should be viewed with suspicion. There are reports of progressive and erosive non-suppurative arthritis of the stifle joints occurring in young cattle, from brucellosis free herds that had been vaccinated with strain-19 vaccine (Radostits *et al*, 2000).

*B. suis* and *B. melitensis* may also infect cattle when they share pasture or facilities with infected pigs, goats or sheep. The infection in cattle caused by heterologous species of *Brucella* may be more transient than that caused by *B. abortus*. However, such cross infections are a serious public health threat, since these *brucellae* which are highly pathogenic to man, can pass into cow's milk. Infection by *B. suis* is not very common but is suspected in contrast, infections by *B. melitensis* have a disease course similar to that caused by *B. abortus* (Acha and Szyfres, 1983).

Bulls are most resistant but may get infection at younger ages or may acquire infection by ingestion. Two clinical pictures are described; one which involves the testis and the epididymis and is characterized by orchitis whereas the other involves seminal vesicular glands and the ampoulae and there may be focal areas of adhesion between tunica vaginalis and the testicle. Sperm granulomas may form with chronic fibrosis of interstitial tissue.

**Goats:** The main etiological agent is *B. melitensis* with its three biotypes. Infections by *B. suis* and *B. abortus* have occasionally been found. The main symptom is abortion, which occurs most frequently in the third or fourth month of pregnancy. Hygromas, arthritis, spondylitis, and orchitis are also observed. In contrast to brucellosis in females of other domestic species, mastitis is a common symptom in goats and may be the first noticeable sign in a flock. Clotting in milk and small nodules in the mammary gland may be observed. In chronically infected flocks the signs of the disease generally are not apparent (Acha and Szyfres, 1983).

**Sheep:** Two disease pictures are distinguishable in sheep: classic brucellosis and ram

epididymitis. Classic brucellosis is caused by *B. melitensis* and constitutes a public health problem equally as or even more important than goat brucellosis. This infection is mostly found in areas with mixed goat and sheep flocks. Sheep are more resistant to infection than to goats and in areas of mixed flocks, fewer sheep than goats are found to be infected.

Ram epididymitis is caused by *B. ovis*. The clinical signs consist of genital lesions in rams associated with varying degrees of sterility. The first reaction in rams is a marked deterioration in the semen quality together with the presence of leukocytes and brucellae. Ram epididymitis is generally unilateral but can be bilateral, and the tail of the epididymis is most commonly affected. The scrotal tunics are usually thickened and hardened and the testicle usually atrophic. Lesions cannot be seen or palpated in most infected rams, even though *B. ovis* may be isolated from their semen. Affected rams have normal libido but will produce semen of lower quality, making them either sub fertile or sterile, depending on the site and severity of the lesion (Radostits *et al.*, 2000).

In the ewe, abortion or birth of weak or stillborn lambs is accompanied by macroscopic placentitis. When localized in the kidneys, *B. ovis* is also shed through the urine (Acha and Szyfres, 1983, Radostits *et al.*, 2000).

**Pigs:** Clinical manifestations of brucellosis in pigs vary but are similar to those seen in cattle and goats. Main causative agent is *B. suis*. After exposure mainly through ingestion of infected tissues and wastes or during service, the pigs develop bacteraemia that may persist for 90 days. During and after the bacteraemia, localization may occur in various tissues. Signs depend considerably on the site(s) of localization. Common manifestations are abortion, temporary or permanent sterility, orchitis, lameness, posterior paralysis,

spondylitis and occasionally metritis and abscess formation in extremities or other areas of the body (Merck's Vet Manual, 1998).

**Horses:** *B. abortus* and *B. suis* have been isolated from these species. The disease usually manifests itself in the form of fistulous bursitis, "poll evil" and "fistulous withers". Abortions are rare. Horses acquire the infection from cattle and swine. Outbreaks of brucellosis in cattle have followed contact with horses with open bursitis (Merck's vet manual, 1998). In general, horses are more resistant to the infection.

**Dogs and Cats:** Sporadic cases of brucellosis caused by *B. abortus*, *B. suis*, and *B. melitensis* occur in dogs. They acquire the disease through eating contaminated material, especially fetuses, afterbirth, and milk. The course of the infection is usually subclinical, but sometimes the symptoms can be severe, with or without fever, emaciation, orchitis, anestrus, arthritis, and at times abortion. The disease is self-limiting and transmission from canine to canine is rare (Acha and Szyfres, 1983).

Brucellosis caused by *B. canis* is characterized by a prolonged afebrile bacteremia, embryonic death, abortions, prostatitis, epididymitis, scrotal dermatitis, lymphadenitis, and splenitis. Abortion occurs about 50 days into gestation. The pups may be still born at term or may die a few days after birth. Survivors usually have enlarged lymph nodes and often have bacteremia (Acha and Szyfres, 1983). Cats are resistant to *Brucella* and no cases of natural disease occurrence are known.

**Other animals:** Brucellosis caused by *B. abortus* occurs in domestic buffaloes, yaks, and camels with symptomatology similar to that in cattle. Most reports of infections with *B. abortus* are in wild herbivores raised together with domestic herbivores in ranches (McDermott and Arimi, 2002, Muma et al., 2006).

Natural infections caused by *Brucella* occur in a wide range of wild species. They have been reported to occur in desert rats (*Neotoma lepida*) of the US, in the European hare (*Lepus europaeus*), the caribou in Alaska, and foxes (*Dusicyon gynocercus*, *D. griseus*) and Grisons (*Galictis furax-huronax*) in Argentina (Acha and Szyfres, 1983).

*Brucella* has been isolated from naturally infected domestic fowl with symptoms varying from weight loss, reduction in egg production, and diarrhea; the course may even be acute. Fowl do not play a role in maintaining the infection in nature. *Brucella* has been isolated from some wild bird species, such as corvids (*Corvus cornix* and *Tripanoscorax fragilecus*) (Acha and Szyfres, 1983).

The recent isolation of distinctive *Brucella* strains, tentatively named *Brucella maris*, from marine animals in the United Kingdom and the United States, extends the ecologic range of the genus and potentially its scope of zoonosis. There is also some evidence of *Brucella* infection in marine mammals in the North Atlantic Ocean (Tryland *et al.*, 2005).

#### **2.1.4.4 Diagnosis of animal brucellosis**

The diagnosis of animal brucellosis is of both public health and economic importance. It signifies a potential source of human infection besides threatening livestock production. The absence of pathognomonic signs in brucellosis may lead to unreliable clinical diagnosis (Hendricks and Meyer, 1975).

Abortion storms in animal populations and individual animals may signify brucellosis (Noakes *et al.*, 2001), and is therefore useful in clinical diagnosis. Presumptive clinical diagnosis based on clinical history of abortion, retention of placenta in the female and lesions in the seminal vesicles and testis in the male must be sustained with the demonstration of the organism and/or specific antibodies in the body fluid for making

confirmatory diagnosis of *Brucella* infection (Chakrabarti, 1993).

While most diagnosticians may use only one of these methods a combination of epidemiology, serology, clinical and bacteriologic evidence would be the best approach (Ramon and Ignacio, 1989). The laboratory techniques are animal inoculation and serologic diagnosis. A review of the test performance for the detection of exposure to *Brucella abortus* and cost comparison shows that the sum of the sensitivity and specificity values for each test was averaged to give a performance index (PI) and allow for a comparison between the different methodologies. A score of 200 was perfect. Based on the PI, the Buffered Antigen Plate Agglutination Test (BPAT) rated highest (PI = 193.1) among the conventional tests. This indicates better accuracy than the other conventional tests including the Rose Bengal test (PI = 167.6) and the complement fixation test (PI = 172.5). Overall, the primary binding assays, including the fluorescence polarisation assay (PI = 196.4), the indirect enzyme-linked immunosorbent assay (PI = 189.8) and the competitive enzyme-linked immunosorbent assay (PI = 188.2), were more accurate than the conventional tests, except for the BPAT. In addition, a fee comparison suggested that the primary binding tests were price competitive with conventional tests for the diagnosis of brucellosis and, therefore, had a better combined cost/efficiency rating. (Gall and Nielsen, 2004)

The serologic diagnostic tests are: - Serum Agglutination Test (SAT), Mercapto-Ethanol test (MET), Complement Fixation Test (CFT), Coomb's Anti-human Globulin Test, Indirect Fluorescent Antibody Test (IFAT), Skin (allergic) test (ST), Rose Bengal Agglutination test (RBT) and Enzyme Linked Immuno-sorbent Assay (ELISA).

#### **2.1.4.5 Treatment of animal brucellosis**

Treatment of animal brucellosis is not normally done and in case it is attempted, it is frequently not successful. In cattle it is unsuccessful because of the intracellular sequestration of the organisms in the lymph nodes, the mammary gland and reproductive organs (Radostits *et al.*, 2000).

Chloramphenicol is reported as successful treatment in infected horses. In rams treatment is economically practicable only in valuable rams and must be instituted before irreparable damage to the epididymis has occurred (Radostits *et al.*, 2000).

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

#### **2.1.5. Brucellosis in man**

Infection with *Brucella* spp. continues to pose a human health risk globally despite strides in eradicating the disease from domestic animals. Brucellosis has been an emerging disease since the discovery of *Brucella melitensis* by Sir David Bruce in 1887. Although many countries have eradicated *B. abortus* from cattle, in some areas *B. melitensis* and *B. suis* have emerged as causes of this infection in cattle, leading to human infections. Currently *B. melitensis* remains the principal cause of human brucellosis worldwide including India. The recent isolation of distinct strains of *Brucella* from marine mammals as well as humans is an indicator of an emerging zoonotic disease. Brucellosis in endemic and non-endemic regions remains a diagnostic puzzle due to misleading non-specific manifestations and increasing unusual presentations. Fewer than 10% of human cases of brucellosis may be clinically recognized and treated or reported. Routine serological surveillance is not practiced even in *Brucella* (Mantur *et al.*, 2007).

*Brucellae* are very invasive, capable of penetrating the mucous membrane of the nose, throat, conjunctiva, urogenital tract, and epithelium of the teat canal, parenchyma of the mammary glands or the testis and normal and abraded skin. Human infection with *brucellae* depends upon contact with infected animals or their products or materials contaminated with animal discharge (Hendricks and Meyer, 1975).

#### **2.1.5.1 Transmission through ingestion of contaminated animal source products**

There is a direct relationship between the level of brucellosis in animal and the human infection, which has been shown to be influenced by methods of husbandry, standards of hygiene and food customs in any community. Consumption of unpasteurised raw milk and dairy products is a common method of transmission (Young, 1983; Cooper, 1991). Raw semi-cooked or pickled meat is a source of human infections (Saddler, 1960). Pasteurisation of milk and heat treatment of meats have reduced brucellosis to an occupational hazard in developed countries; however this is not the case in Kenya.

Milk from domestic animals and water buffaloes is the most common source of infection, with *Br. melitensis* being more easily transmissible by this method than *Br. abortus* (Flores-Castro and Baer, 1979). Dairy products like Cheese, cream, butter, and yoghurt prepared from unpasteurised milk are a good source of infection (CDC, 1976), with the cream fraction being more heavily laden with the organisms than the skimmed fraction (FAO/WHO, 1971), thus requiring more heating to kill the organisms.

*Brucellae* can survive pickling, smoking, and freezing of meats. Occasionally contamination may result from vegetables and water coming into contact with infected discharge, secretion, or animal excreta (FAO/WHO, 1971; Ray, 1979)



### **2.1.5.2 Transmission through inhalation**

Inhalation of infected dried materials of animal origin in houses, laboratories, abattoirs and farm premises used for housing animals may lead to infection through the respiratory route and conjunctiva (FAO/WHO, 1971). Infection can follow inhalation of contaminated aerosols or dusts. Airborne transmission of brucellosis has been studied in the context of using brucella as a biological weapon. In fact, *B. suis* was the first agent contemplated by the U.S Army as a potential biologic weapon (Smart, 1997) and is still considered in that category. In a Hypothetical attack scenario, it was estimated that release of an aerosolized form of brucella under optimal circumstances for dispersion would cause 82,500 cases of brucellosis and 413 fatalities (Kaufmann et al., 1997). Cases of laboratory- acquired brucellosis are the perfect examples of airborne spreading of the disease (Eurgonul et al., 2004). A premature infant with transplacentally acquired congenital brucellosis and pulmonary involvement has been described (Koklu et al., 2006, Singer et al., 1991)

### **2.1.5.3 Other minor routes**

*B. melitensis* has been isolated from vaginal mucosa, urine and milk of infected women and semen of infected men (Vandercamp et al., 1990). This does not constitute any evidence to implicate venereal transmission, though it can occur in some circumstances. Ruben et al (1991), observed transmission of *B.melitensis* between spouses in what was suggested as person to person transmission through coitus. There are isolated reported cases of transmission through blood transfusion( Doganay et al., 2001), bone marrow transplantation (Ertem et al.,2000) and to nursing babies through mother milk (Celebi et al. 2007). Transplacental transmission is another potential mode but seems unlikely as human placenta lacks erythritol (Poulou et al., 2006). Blood sucking arthropods have

been shown to harbour the organisms which multiply and persist in both ticks and insects and still remain virulent to man. Arthropods may also transmit through bites while ticks may transmit through contact as they excrete the organisms in coxal fluid though there is little evidence of their direct role in natural transmission. Mosquitoes and flies can be infected experimentally, but it is doubtful whether transmission occurs naturally through insect bites (Radostits *et al.*, 2000).

#### **2.1.5.4 Brucellosis as an occupational hazard**

The relative importance of mode of transmission and pathway of penetration of the etiological agent varies with the epidemiologic area, the animal reservoirs, and the occupational groups exposed to the risk (Acha and Szyeres, 1983). Brucellosis is an occupational disease in shepherds, abattoir workers, veterinarians, dairy industry professionals, and personnel in microbiologic laboratories. One important epidemiologic step in containing brucellosis in the community is the screening of household members of infected persons (Pappas *et al.*, 2005. Zachou *et al.*, 2008).

There is limited evidence of person-to-person transmission of *Brucella* spp. and might only occur via the urine or blood transfusion. Venereal transmission has been reported between a laboratory worker and his spouse. *B. melitensis* abscesses in a woman's breast may serve as a source of infection for her infant. Human disease prevalence in any given area closely parallels animal prevalence, although the likelihood of disease in humans is further greatly influenced by the degree of contact with animals or their excreta, and especially by ingestion of unpasteurized milk or inadequately cooked meat from infected animals (Mantur, 2007).

### 2.1.5. 5. Pathogenesis and clinical disease

Brucellosis is a systemic disease that can involve almost any organ system. Infection through the skin causes a more severe and rapid reaction than, when the bacillus is ingested or inhaled (Radostits et al, 2000).

Upon penetrating the epithelial barriers, the organisms are ingested by neutrophils and tissue macrophages, which, in turn, transport them to local lymph nodes. Bacteremia develops within 1-3 weeks of exposure if the host immune system cannot contain the infection. Organisms then localize in the organs of the reticuloendothelial system, primarily the liver, spleen, and bone marrow, where the formation of granulomas ensues. Large granulomas serve as a source for persistent bacteremia (Lisgaris, 2005).

The primary virulence factor for *Brucella* species is the cell wall lipopolysaccharide (LPS). Both smooth (e.g. *B. melitensis*, *B. abortus* and *B. suis*) and rough forms (*B. canis*) exist with strains displaying rough LPS that have much less virulence in humans. After opsonization and ingestion by phagocytic cells, organisms can be maintained intracellularly within phagosomes. Susceptibility to intracellular killing differs between species, *B. abortus* is readily killed and *B. melitensis* rarely affected. Serum lysis occurs, even in the absence of agglutinating antibodies, with *B. abortus* being much more susceptible to lysis than *B. melitensis* ( Lisgaris, 2005).

Infection in human may remain latent or subclinical or it may give rise to symptoms of varying intensity and duration. Brucellosis can present as an acute or sub acute pyrexial illness, which may persist for months or develop into focal infection that can involve almost any organ system. The characteristic intermittent waves of elevated temperature are usually seen in long standing untreated cases (Corbel, 2002).

The incubation period is generally 1-2 months, after which the onset of illness may be acute or insidious. Symptoms of brucellosis are protean in nature, and none are specific enough to make the diagnosis. Thereafter, symptoms may include: an intermittent, "undulating" fever, headaches, chills, depression, profound weakness, arthralgia, myalgia, weight loss, and orchitis/epididymitis in men and spontaneous abortion in pregnant woman (OIE/CFSPH, 2004).

Brucellosis lasts for days to months, and can be quite debilitating, although the case fatality rate is very low (except in cases of *B. melitensis* endocarditis). Chronic sequelae may include sacroiliitis, hepatic disease, endocarditis, colitis and meningitis (Olsen, 2004).

#### **2.1.5.6 Diagnosis of brucellosis in man**

The signs and symptoms of brucellosis are not specific. Pointers to the diagnosis are a history of occupational exposure or recent travel to endemic areas with consumption of milk products (Corbel, 2002).

#### **2.1.5.7 Laboratory diagnosis**

Brucellosis is confirmed in man by isolating the organism from blood, cerebrospinal fluid (CSF), peritoneal fluid, semen, vaginal swabs and tissues from the spleen, liver and lymph nodes, which are specimens of choice (Kelly et al, 1960.)

When brucellosis is suspected, blood culture should be attempted repeatedly, although 30-50% may show negative results. *B. melitensis* and *B. suis* are more readily isolated from blood than *B. abortus*. The organism is cultured for 7 and 21 days but should only be declared negative after 45 days incubation (Rodriguez-Torres, 1983 quoted by Yagupsky

1999). *B. mellitensis* may also be isolated in urine samples. In the absence of positive cultures, the diagnosis of brucellosis usually depends on serological tests, the results of which tend to vary with the stage of the infection. The serological tests include Standard agglutination test, Rose Bengal plate test, Mercaptoethanol test, Complement fixation test and ELISA among other tests (Corbel, 2002). 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. Promising results have been obtained in clinical studies (Corbel, 2002)

Diagnosis of brucellosis requires prompt detection and identification of coccobacillus for appropriate patient management as the organism is associated with a potentially severe outcome (Zimmerman *et al.*, 1990). Isolation of the organism by culture or guinea pig inoculation is done from citrated blood. However, isolation from blood is not always possible and serological tests play a major role in the routine diagnosis.

The routine serologic tests offer good results for the diagnosis of brucellosis upon use of adequate cut-off points (Lambea *et al.*, 1992; Moreno *et al.*, 1992). Some researchers have, however, highlighted limitations of these serological tests. Hornitzky and Searson (1986) isolated *brucellae* from animals that were serologically negative to Rose Bengal, serum agglutination and complement fixation tests.

Serological tests applied in human beings are modifications of those used in animals.

The stimulation of the immune system by brucella antigens shows a transient character (Vendrell *et al.*, 1992). Immunoglobulin G (IgG) and/ or IgM/IgA antibodies are secreted by peripheral blood mononuclear cells. They disappear 5 to 20 months after onset of clinical signs and 20 to 27 days after vaccination. Detection of these immunoglobulins

could improve the diagnosis of brucellosis. Serological tests used include:

#### **2.1.5.7.1 Tube Agglutination Test (TAT).**

The test detects specific and non-specific antibodies from *Brucella* infection and vaccination. This test is laborious but it can be used as a basic diagnostic tool to confirm result obtained with other serologic methods, such as the plate test. Non-specific reactions are less frequent than with the plate test (Alton, 1975).

Tube agglutination test has a low sensitivity as shown by some studies, 61% and 69% by Dohoo *et al.*, (1986). The test is used in serological screening but it may not effectively detect positive patients in either the acute or chronic stages of the disease (FAO/WHO, 1971). However, Moreno *et al.*, (1992) found that at an adequate cut-off point of 1/160, the test had a sensitivity of 93% and a specificity of 97%. This specificity estimate was in close agreement with that of Dohoo *et al.* (1986) who reported 99% specificity.

#### **2.1.5.7.2 Plate Agglutination Test (PAT).**

The plate test has been widely and successfully used in the United States of America as a simple and rapid laboratory test (Alton, 1975). It can be applied singly or in conjunction with the tube test. Suspicious and positive sera to the plate test are submitted to the slow tube test (plate test is incubated at 37°C for 1hr 30 minutes, while the tube test is incubated overnight at the same temperature). As the test is rapid, it can be used in surveys to establish the prevalence of the disease.

It is affected, to a lesser extent than the tube test, by the presence of incomplete antibodies that bring about prozone phenomenon, and serum hemolysis (Alton, 1975), but

shows non-specific agglutination. Incomplete antibodies are those which even though they combine with the antigen, do not produce the second phase of the reaction i.e. agglutination, and generally belong to the IgG class. Using standardized antigen, the plate test is, within narrow limits, equal in sensitivity to the United States Department of Agriculture (USDA'S) TAT (Alton, 1975) and Dohoo *et al.*, (1986) also recorded similar sensitivities between the TAT and PAT.

#### **2.1.5.7.3 The Rose Bengal Plate Test (RB PT).**

The RBPT is also called the buffered antigen or the *Brucella* side test. The Rose Bengal test consists of Rose Bengal-stained *Brucella* spp. antigen and agglutinates *Brucella* spp-specific antibodies in human or animal serum. Thirty microliters of serum and Rose Bengal test solution were mixed on a slide and agglutination was evaluated after 3 minutes (Steinmann *et al.* 2006). This is a simple, rapid test which detects early infection and can be used as an initial screening test (FAO/WHO, 1971; Hossain *et al.*, 1991; Moreno *et al.*, 1992). The test varies considerably in terms of sensitivity and specificity of 96%. The work of Dohoo *et al.* (1986) has shown a specificity of 100% and a sensitivity of 74.9%. Moreno *et al.* (1992) have recorded high sensitivity value of 95% and a low specificity value of 75%.

RBPT can give false positive results with sera from patients having *Yersinia enterocolitica* 0:9 infection or healthy patients exposed to smooth *Brucella* (Russel *et al.*, 1978).

#### **2.1.5.7.4 The Card Test (USA)**

This is a rapid agglutination test that employs a Rose Bengal stained antigen buffered at

pH 3.65 and adjusted to contain 8% of cells by volume, as determined by the packed cell volume method. It is also used for screening purposes and indicates the presence or absence of *Brucella* IgG agglutinins. Its sensitivity and specificity agrees with that of the RBPT (Se= 74.3%, Sp= 100%) (Alton, 1975).

#### **2.1.5.7.5 Mercaptoethanol Test (2MET).**

This test is an agglutination test carried out in presence of 2-mercaptoethanol, which inactivates IgM molecules present in the serum being tested (Anderson *et al.*, 1964). Thus, the test is an indicator of anti-*Brucella* IgG agglutinin present in the serum, and a positive titre indicates active infection (Alton, 1975). It is recommended that the test be performed simultaneously with the standard tube test. Though its sensitivity is low (59.9%, Dohoo *et al.*, 1986), it is very important in human brucellosis diagnosis especially of chronic cases and should be performed as a matter of routine (Alton, 1975).

#### **2.1.5.7.6 The Coombs (antihuman-globulin) Test**

The test was developed by Coombs *et al.* (1945). It is both sensitive and specific for the diagnosis of brucellosis but is laborious. The Coombs test utilizes the Coombs reagent to bring about agglutination in the presence of incomplete antibodies. The Coombs reagent is an antiserum specific against either globulin or whole serum. The test is particularly useful in diagnosis of chronic brucellosis, where a positive Coombs test titre may be present when the ordinary agglutination titre is either low or absent. It is very sensitive and detects exposed individuals such as veterinarians and laboratory workers who are symptomless. At a cut-off of 1/320 the test has a sensitivity of 92% and a specificity of 100% (Moreno *et al.*, 1992).



#### **2.1.5.7.7. Complement Fixation Test (CFT)**

The CFT rarely exhibits non-specific reactions. The CFT titres do not wane as the disease becomes chronic and often they reach diagnostic levels sooner than the serum tube agglutination test following natural infection (Alton, 1975). The test has advantages over seroagglutination. Drawbacks on serum agglutination test include the inability to diagnose *B. canis* infections; the appearance of cross-reactions of class M immunoglobulins with *Francisella tularensis*, *Escherichia coli* 0116 and 0157, *Salmonella urbana*, *Yersinia enterocolitica* 0:9, *Vibrio cholerae*, *Xanthomonas maltophilia*, and *Afipia clevelandensis* and the percentage of cases in which seroconversion does not occur. Lack of seroconversion can be attributed to the performance test early in the course of infection, the presence of blocking antibodies, or the so-called “prozone” phenomenon ( the inhibition of agglutination at low dilutions due to an excess of antibodies or to non specific serum factors)(Young, 1991).

#### **2.1.5.7.8 Enzyme Linked Immunosorbent Assay (ELISA) Test.**

This test can be used as a supplementary test to the CFT. It has been found to be more sensitive, economical and rapid than other tests in screening humans for brucellosis under field conditions. Dot and plate ELISA methods for the detection of human IgG and IgM *Brucella* antibodies were compared with agglutination and complement fixation tests by Mongini *et al.*, (1990). Comparison of these two methods with a standard serological test kit indicated that both tests were sensitive and specific. Similar work by Hornitzky and Searson (1986) has shown ELISA to be superior to RBT and CFT. ELISA is also capable of successfully differentiating acute and chronic brucellosis (Araj *et al.*, 1986; Saj *et al.*,

1987; Lulu *et al.*, 1988). ELISA is the most sensitive and reliable diagnostic test especially in relation to chronic brucellosis and neurobrucellosis. It allowed determination of IgG, IgM and IgA in cerebrospinal fluid, and provided profiles of immunoglobulin in sera from patients with chronic form (elevated IgG and IgA) which were different in patients with acute form of brucellosis (elevated IgM alone or IgG, IgM and IgA) (Lulu *et al.*, 1988; Janbon, 1993).

The diagnostic methods now commonly used for human serological testing are agglutination tests and the complement fixation test (CFT). Among the newer serological tests, primary binding assays were developed to improve sensitivity and specificity. The indirect enzyme immunoassay (IELISA) appears to be the most sensitive; however, interpretation may be difficult, as false-positive reactions may occur due to exposure to, for instance, *Yersinia enterocolitica* O: 9 (WHO, 1993). Another problem with the IELISA is the standardization of reagents, which should be improved in order to make inter-laboratory results easy to interpret (WHO, 1993; Young, 1989). The competitive enzyme immunoassay (CELISA) for the detection of serum antibody to *Brucella* is a multi-species assay, which appears to be capable of differentiating vaccinal antibodies from antibodies elicited by field infection in cattle (Nielsen *et al.*, 1995). The monoclonal antibody used in this assay is specific for a common epitope of smooth lipopolysaccharide (S-LPS). The test is fairly rapid but does require some equipment or manipulation.

#### **2.1.5.7.9 Allergic Tests.**

Hypersensitivity to *brucella* antigens is acquired during the course of infection, following vaccination or following exposure to the organisms or killed antigens in the laboratory. A Positive allergic test indicates invasion of the tissues by *Brucella*, but does not prove the presence of active disease (Darlmpole-Champneey, 1960; Alton, 1975). The chief value of the test is for epidemiological purposes.

#### **2.1.5.7.10. PCR (Polymerase Chain Reaction)**

Brucellosis is a worldwide disease of humans and livestock that is caused by a number of very closely related classical *Brucella* species in the alpha-2 subdivision of the Proteobacteria. Halling et al., (2005), compared the complete genome sequence of *Brucella abortus* field isolate 9-941 to those of *Brucella suis* 1330 and *Brucella melitensis* 16 M. The genomes of these *Brucella* species are strikingly similar, with nearly identical genetic content and gene organization. However, a number of insertion-deletion events and several polymorphic regions encoding putative outer membrane proteins were identified among the genomes. Several fragments previously identified as unique to either *B. suis* or *B. melitensis* were present in the *B. abortus* genome. Even though several fragments were shared between only *B. abortus* and *B. suis*, *B. abortus* shared more fragments and had fewer nucleotide polymorphisms with *B. melitensis* than *B. suis*. The complete genomic sequence of *B. abortus* provides an important resource for further investigations into determinants of the pathogenicity and virulence phenotypes of these bacteria (Halling et al. 2005)

. PCR is fast, can be performed on any body tissue, and can yield positive results as soon

as 10 days after inoculation. It was first developed for brucellosis in 1990, using a 635-bp fragment of *B. abortus* strain 19 (Fekete et al., 1990). Subsequently, two major gene sequences have been used as targets: the 16S rRNA gene sequence, (Romero et al., 1995) which presents total gene-specific homology and has been satisfactory in clinical setting, (Nimri et al 2003) and the *BCSP31* gene, which encodes an immunogenic protein of the external membrane of *B. abortus* (Baily et al., 1992) and has been extensively studied in clinical practice (Morata et al., 2002). A comparison of the two techniques showed superiority of the 16SrRNA target in terms of sensitivity (Navarro et al., 2002).

Nested PCR has proved to have superior specificity and sensitivity, although it is more prone to contamination (Matar et al., 1996). Real time PCR is most likely the diagnostic tool of the future, offering the possibility of results in 30 minutes (Redkar et al., 2001, Probert et al, 2004, Queipo-Ortuno et al., 2005). PCRELISA is another new promising variation (Vrioni et al., 2004, Morata et al., 2003). Other variations of PCR exist, such as arbitrarily primed PCR, PCR with random amplification of Polymorphic DNA, and a specific multiplex PCR that can concomitantly diagnose brucellosis, Q fever, plague, anthrax and was developed for purposes of biological warfare defence (McDonald et al., 2001). Although PCR is very promising, standardisation of extraction methods and set-up is lacking, and a better understanding of the clinical significance of the results is still needed (Navarro et al., 2004).

## 2.2 MALARIA

### 2.2.1 Definition

Malaria is a serious and sometimes-fatal disease caused by *plasmodium*. Patients with malaria typically are very sick with high fevers, shaking chills, and flu-like illness. Four kinds of malaria parasites can infect humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*.

Benign malaria is commonly contracted in the subtropics and temperate zones rather than near the equator. It has a marked tendency to subsequent relapses. Malignant (tropical) malaria is contracted in the true tropics and sub-tropics. This infection is associated with the severest bouts of malaria, which are life threatening, particularly in children and pregnant women (Zucker *et al.*, 1995, Bulmer *et al.*, 1993). Epidemics, especially during the wet season, can affect large proportion of the population.

Infection with any of the malaria species can make a person feel very ill; infection with *P. falciparum*, if not promptly treated, may be fatal. Although malaria can be a fatal disease, illness and death from malaria are largely preventable.

### 2.2.2 Transmission of malaria

Usually, people get malaria by being bitten by an infected female *Anopheles* mosquito. Only female *Anopheles* mosquitoes can transmit malaria and they must have been infected through a previous blood meal taken from an infected person. When a mosquito bites, a small amount of blood is taken in which contains the microscopic malaria parasites. The parasite grows and matures in the mosquito's gut for a week or more, then travels to the mosquito's salivary glands. When the mosquito next takes a blood meal, these parasites mix with the saliva and are injected into the bite (CDC 2007).

Once in the blood, the parasites travel to the liver and enter liver cells to grow and multiply. During this "incubation period", the infected person has no symptoms. After as few as 8 days or as long as several months, the parasites leave the liver cells and enter red blood cells. Once in the cells, they continue to grow and multiply. After they mature, the infected red blood cells rupture, freeing the parasites to attack and enter other red blood cells. Toxins released when the red cells burst are what cause the typical fever, chills, and flu-like malaria symptoms.

If a mosquito bites this infected person and ingests gametocytes the cycle of transmission continues. (CDC 2007). Because the malaria parasite is found in red blood cells, malaria can also be transmitted through blood transfusion, organ transplant, or the shared use of needles or syringes contaminated with blood. Malaria may also be transmitted from a mother to her fetus before or during delivery ("congenital" malaria).

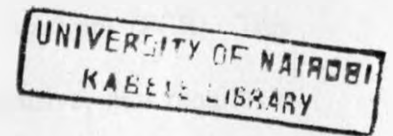
### **2.2.3 Diagnosis of malaria**

#### **2.2.4.2 Blood smear**

Malaria parasites can be identified by examining under the microscope a drop of the patient's blood, spread out as a "blood smear" on a microscope slide. Prior to examination, the specimen is stained (most often with the Giemsa stain) to give to the parasites a distinctive appearance. This technique remains the gold standard for laboratory confirmation of malaria. However, it depends on the quality of the reagents, of the microscope, and on the experience of the technician.

#### **2.2.4.3 Antigen detection**

Various test kits are available to detect antigens derived from malaria parasites. Such



immunologic ("immunochromatographic") tests most often use a dipstick or cassette format, and provide results in 2-10 minutes. These "Rapid Diagnostic Tests" (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available. Malaria RDTs are currently used in some clinical settings and programs. However, before malaria RDTs can be widely adopted, several issues remain to be addressed, including improving their accuracy; lowering their cost; and ensuring their adequate performance under adverse field conditions. Malaria RDTs are currently not approved by the U. S. Food and Drug Administration (FDA) for use in the United States. The World Health Organization's Regional Office for the Western Pacific (WHO/WPRO) provides technical information, including a list of commercially available malaria RDTs, at (WHO 2005).

#### **2.2.4.4 Molecular diagnosis**

Parasite nucleic acids are detected using polymerase chain reaction (PCR). This technique is more accurate than microscopy. However, it is expensive, and requires a specialized laboratory. Technical advances are however likely to result to field-operated PCR machines.

## **2.3 TYPHOID**

### **2.3.1 Etiology**

Typhoid fevers are infections by *Salmonella typhi* or one of the three paratyphoid bacilli, *Salmonella paratyphi A*, *B* and *C*. The ultimate source of infection is patients suffering from the disease, or the excreta of carriers. The main vehicle of transmission is contaminated water supplies, food and air (Yew *et al.*, 1993). Flies and infected dust may

be the sources of infection, especially in endemic areas (Christian, 1938 quoted by Huckstep, 1962).

### **2.3.2 Signs and symptoms of typhoid fever**

Symptoms include abdominal discomfort, back pains, diarrhoea without blood, a slight cough and minimal vomiting. General malaise, fever, anorexia, headache and shivering are general symptoms (Huckstep, 1962; Endeshaw and Habte, 1987), but according to Tumwine (1983), fever and anorexia, along with diarrhoea and vomiting are common symptoms. On examination the typhoid fever patient is lethargic, has dry skin and lips, a musty odour and furred tongue, slow pulse, enlarged spleen and flat spots and is feverish. The only way to know for sure if an illness is typhoid fever is to have samples of stool or blood tested for the presence of *S. typhi*. Confusion, delirium, intestinal perforation, and death may occur in severe cases. The etiologic agent may be recovered from the bloodstream or bone marrow, and occasionally from the stool or urine.

### **2.3.3 Diagnosis of typhoid fever in Man**

Two techniques are used. These are culture and serotyping of the salmonella colonies by slide agglutination.

#### **2.3.3.1 Widal Test**

When facilities for Culturing are not available, the Widal test is performed and interpreted with care; it can be of value in the diagnosis of typhoid fever in endemic areas. The patient's serum is tested for the O and H antibodies against the following antigen suspensions. (Usually stained suspensions); *S. Typhi* O 9, 12 suspension and *S.Typhi* H d suspension.



Salmonella antigens suspensions are commercially available in 5ml amounts from Wellcome Reagents Ltd and other manufacturers. The antigens are suitable for rapid slide (screen) testing and tube testing. Before use, the suspensions must be allowed to warm to room temperature and be well mixed. The test must be well controlled.

Sufficient serum for the Widal test can be obtained from 3-5ml of patient's blood collected into a clean dry tube and allowed to clot. The serum should be free from red blood cells and must not be heated.

The Widal test is reported by giving the titre for both the O and H antibodies. The antibody titre is taken as the highest dilution of serum in which agglutination occurs. The type of agglutination seen with O reactions is granular while that seen with H reactions is a more uneven type of clumping usually described as floccular. Both slides and tube tests are more easily read against a dark background. If no agglutination occurs the test should be reported as;

*S.typhi* O titre less than 1 in 20,

*S. typhi* H titre less than 1 in 20'

### **2.3.3.2 Culture**

The other technique is blood culture for isolation and identification of *Salmonella typhi*. See section 3.2.6 page 42.

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Study area**

The study was conducted in Kibera; the largest urban slum centre in East Africa. Kibera is located in the South of the capital, Nairobi. The common diseases in man include malaria, typhoid, cholera and HIV/AIDS. Most of the inhabitants live below the poverty line with the slum lacking a functioning sanitation and drainage system. Most people living in Kibera are migratory in nature, moving between upcountry and the slum area.

#### **3.2 Methodology**

##### **3.2.1 Study sites**

The study took place at AMREF Kibera community Clinic, and Mbagathi District Hospital.

##### **3.2.2. Clearance to undertake research**

This involved the acquisition of the authority to carry out the research from the Ministry of Education Science and Technology, Ethical clearance from the National Council for Science and Technology and authority to carry out research in health facilities in the City of Nairobi from the Medical Officer of Health (See appendix 6.1, 6.2 and 6.3 for authorizations)

### **3.2.3. Study patients' recruitment.**

Patients presenting with signs consistent with brucellosis were singled out to participate in the project. Their clinical history was taken and patients examined for: cough, diarrhea, vomiting, fever, joint pains, neuropsychiatric symptoms, lymphadenopathy, hepatomegaly, and splenomegaly). The study was explained and each willing patient signed a consent form and was registered for the study.

### **3.2.4. Blood Sampling**

Blood sample was collected via antecubital fossa in strict aseptic method. The venepuncture site was meticulously disinfected using 70% alcohol (preferably in combination with 10% Povidone Iodine). The disinfectant was then allowed to evaporate from the skin surface and 10ml of blood was withdrawn. Blood collected was divided into two portions. One portion was used to culture *S. typhi* and the other for separating serum for serological tests.

### **3.2.5 Serological Tests for Brucellosis**

To obtain serum, blood was allowed to clot then spun in a centrifuge and the serum removed and placed in 1ml cryovials at the hospital laboratory. Brucella test Kits manufactured by Eurocell diagnostics Limited , Biotec laboratories, UK; Tulip diagnostic (P) Ltd India and Fortress diagnostics limited UK; which are commonly used in laboratories to diagnose brucellosis were purchased from local suppliers. These are all slide agglutination Kits and work on the same principle as Rose Bengal Plate test. Competitive ELISA and Rose Bengal plate tests were purchased from Veterinary laboratory agency, UK.

Serum samples tested using ELISA, RBPT were all subjected to these rapid tests.

### 3.2. 5.1 Competitive ELISA

Detection of antibodies against *brucella* in serum samples was done using COMPELISA kit purchased from Veterinary Laboratory Agency UK. Manufacturer's recommendation on storage and reagents preparation were followed. Conjugate solution was prepared and diluted to working strength with diluting buffer according to instructions on the ampoule label. Twenty microliters of each test serum was added to wells of a pre-coated plate with brucella antigen while leaving columns 11 and 12 for controls. Each test was done in duplicate. Twenty microliters of the negative control was added to wells A11, A12, B11, B12, C11 and C12, while 20 $\mu$ l of the positive control was then added to wells F11, F12, G11, G12, H11 and H12.

The remaining wells (D11- D12 and E 11- E 12) having no serum added acted as the conjugate controls. One hundred microliters of the prepared conjugate solution was immediately dispensed into all the wells. This gave a final serum of 1/6. The plate was then vigorously shaken (on the microtitre plate shaker) for 2 minutes in order to mix the serum and conjugate solution. The lid was covered and the plate incubated at room temperature ( $21^{\circ}\text{C} \pm 6^{\circ}\text{C}$ ) for 30 minutes on a rotary shaker, at 160 revs/min.

The contents of the plate were then carefully poured out and washed 5 times with washing solution and then thoroughly dried by tapping on absorbent paper towel. A microtitre plate reader was switched on and the unit allowed to stabilize for 10 minutes. Immediately before use the substrate and chromogen solution was prepared by dissolving one tablet of urea  $\text{H}_2\text{O}_2$  in 12 ml of distilled water. When dissolved the opd (O - phenylenediamine Dihydrochloride 10mg) tablet was added and mixed thoroughly. And

100 µl was added to all wells. The plate was left at room temperature for a minimum of 10 minutes and a maximum of 15 minutes.

The reaction was stopped by adding 100µl of stopping solution to all wells. Condensation was removed from the bottom of the plate with absorbent paper towel and the plate read at 450 nm. If a microtitre plate reader is not available a visual inspection of the plate may be used to determine whether a sample is positive or negative. The lack of colour development indicates that the sample tested was positive. The Positive or negative cut – off was calculated as the 60% of the Mean optical Density (OD) of the four conjugate control wells. Any test sample giving an OD equal to or below this value was regarded as being positive.

#### **3.2.5.2 Rose Bengal Plate Test**

The reagent and samples were allowed to reach room temperature. The well tile was labelled with specimen number. Thirty microlitres of the sample (serum) was added to each well. The RB reagent was swirled gently before drawing 30µl which was added onto the serum specimen. The two were mixed well with applicator stick and placed on a rotator at 100rpm for not more 4 minutes

The wells were examined macroscopically for the presence or absence of visible agglutination immediately after removing the slide rotator. The presence of agglutination indicated an antibody and therefore brucella positive while no agglutination represented brucella negative samples respectively.

#### **3. 2.6 Blood culture for *Salmonella typhi***

Laboratory analysis of all the blood samples collected were partly conducted at the

AMREF Kenya country office microbiology laboratory and partly at the University of Nairobi's Department of Public Health, Pharmacology and Toxicology, immunology laboratory with the exception of the Rose Bengal plate test and the other tests that are used at the clinics to screen patients for brucellosis and the stained blood film examination for malaria parasites.

The portion of the blood for culture was inoculated into sterile tryptone soy broth. The bottle caps were sterilized with surgical spirit before inoculating blood into culture bottles; a new sterile needle was used. Tryptone soy broth and blood were then thoroughly mixed to prevent clotting. The bottles were labelled with the names of the patient/ laboratory number and date of collection and transported to AMREF central laboratory for incubation at 35-37°C, isolation and identification of *S. typhi*.

### **3.2.7 Stained blood film examination for malaria parasites**

#### **3.2.7. 1 Principle and purpose**

Blood parasites stained with Romanowsky dyes (see appendix 6.10) are identified morphologically by microscopic examination. The Romanowsky stain is the preferred choice for routine use is field stain. Thick blood films are used for the detection of parasites, and thin blood films are used for more detailed morphological examination to correctly identify the parasites to species level

#### **3.2.7.2. Thick blood films**

A thick film was made by placing a drop of blood on a horizontal glass slide and allowing it to dry at room temperature away from direct sunlight and hot objects. The thick blood film was stained using the field stain technique by dipping in field stain A

solution (see appendix 6.10) for 4 seconds, in water for 5 seconds, followed by dipping in field stain B (see appendix 6.10) for 4 seconds and washing in water for 5 seconds after which they were dried. A drop of immersion oil was placed on the thick film and viewed under the microscope at x10 for large parasites.

The thick film was then viewed under x100 (oil immersion) objective systematically. The trophozoite ( *Plasmodium falciparum* (ring forms, not gametocytes)), and white blood cells in each field were counted. The number of parasites and white cells were counted separately in the various fields. This was done to include fields with either no parasites or white cells, until the total white blood cell (WBC) count reached 100. Parasite counting was restricted to infection with *P. falciparum* malaria.

### **3.2.7.3. Thin blood film**

The thin film was made by obtaining a drop of blood which was then spread using a spreader and was then waved in air to dry immediately after preparation. The thin film was then fixed in absolute alcohol for 2 seconds and allowed to dry. The slide was stained using the reverse Field stain technique by dipping in field stain A for 6 seconds and washing in water for 5 seconds.

The thin film was examined to count malaria parasites accurately should they be too numerous to be counted on the thick film; to confirm the parasite species (if there are several parasite stages present e.g. trophozoites and schizonts, or with two nuclei) and to confirm any haematological abnormalities detected in thick films. A drop of immersion oil was placed on the tail of the thin film and examined with the x100 (oil immersion) objectives. An area where the red cells do not overlap is selected. Infected and uninfected

red cells in five fields are counted using two hand tally counters and the percentage of infected red cells determined.

If species other than *P. falciparum* were suspected, the tail or edges of the film were examined and identity of parasite species noted by the following features: - shape of trophozoites in a red cell; number of trophozoites in a red cell; size of infected red cells; other inclusions in infected red cells; number of merozoites in a schizont and shape of gametocytes. In *P. vivax* and *P. ovale* infections, parasites may be scanty in peripheral blood. In suspected infections several thick and thin blood films were made and examined carefully. Table 2 below shows the tests carried out and the number of samples in each case.



**Table 1 : Tests performed on the 105 blood samples collected**

<b>Test</b>	<b>Number of samples tested</b>
Competitive ELISA	86
Rose Bengal Plate Test	86
Biotech	86
Eurocell	86
Fortress	86
Tulip	86
Blood slide smear	105
Blood culture for S.typhi	105

All the samples collected were tested for malaria and typhoid. Not all the samples were subjected to brucellosis test because the serum collected from each sample was of different quantities due to the different hematocrit content in the individuals blood.

### **3.3 Data entry and analysis**

All data was entered and managed in MS Excel. The data was then opened in Instat<sup>R</sup> Statistical software for generation of descriptive statistics.

## CHAPTER FOUR

### 4.0 RESULTS

Different test kits are readily available in the market for the detection of antibodies against *brucella*, but all of them work on the principle of agglutination just like Rose Bengal plate test; they are the ones found in different hospital laboratories.

In this study, blood culture was preferred to Widal test in the detection of *S. typhi* to reduce the possibility of getting false positives; although this was not routinely done in the hospital set up where widal tests are usually done, because the test kits are readily available and are easier and faster to use. Blood smears are routinely carried out in our health facilities as the standard test for the detection of malaria.

### 4.1 Clinical examination of patients

A total of 105 patients were recruited to the study during the nine months period that sample collection was carried out. Patients that were tested for Brucellosis showed a combination of signs as shown in Table 2. Most patients complained of headaches, fever, joint pains, backaches, abdominal discomfort, general malaise, chills and coughs. Most male patients reported to have fever, joint pain, and abdominal discomfort and chill whereas most female patients exhibited backaches, general malaise and coughs

**Table 2: Summary of signs and symptoms reported by the 105 patients attending clinic by gender**

Symptoms			
	Total	Female (%)	Male (%)
Headache	54	27 (50)	27 (50)
Fever	51	22 (43)	28 (57)
Joint pain	41	19 (46)	22 (54)
Abdominal discomfort	23	9 (39)	14 (61%)
General malaise	22	14 (64)	7 (36)
Backache	33	19 (56)	14 (44)
Cough	13	7 (54)	5 (46)
Chills	23	9 (43)	13 (57)
dizziness	6	3 (50)	3 (50)
Chest pain	7	6 (86)	1 (14)
Nausea	2	2 (100)	0 (0)
Night sweats	2	0 (0)	2 (100)
Neck pain	2	1 (50)	1 (50)
Lumbar/lumbosacral pain	2	0 (0)	2 (100)
Vomiting	1	1 (100)	0 (0)
Fatigue	1	1 (100)	0 (0)
Shoulder pain	1	0 (0)	1 (100)
Abdominal distension	1	1 (100)	0 (0)
Palpitations	1	1 (100)	0 (0)
Rash	1	1 (100)	0 (0)

Laboratory tests carried out on the samples included; test for brucellosis, blood slide smear test for malaria and blood culture for *Salmonella typhi*. The findings indicate that only one person tested positive for malaria while other two different people were positive for typhoid. These samples that tested positive for malaria and typhoid were negative for brucellosis.

**Table 3 Summary of the laboratory results**

Test	Samples tested	Samples testing positive
Competitive ELISA for brucellosis	86	0
Rose Bengal Plate Test for brucellosis	86	1
Biotech test for brucellosis	86	25
Eurocell test for brucellosis	86	8
Fortress test for brucellosis	86	29
Tulip test for brucellosis	86	14
Blood slide smear for malaria	105	1
Blood culture for S.typhi	105	2

## 4.2 Distribution of *B. abortus* and *B. mellitensis*

**Table 4 : Comparison of the results of Brucellosis obtained by Competitive ELISA, Rose Bengal Plate Test and commercially available agglutination tests kits**

Test	Total samples	No. Positive	Positive with <i>B.abortus</i> antigen	Positive with <i>B. mellitensis</i> antigen	Positive for both <i>B. abortus</i> and <i>B.mellitensis</i> antigens
Competitive ELISA	86	0	-	-	-
Rose Bengal	86	1	-	-	-
Biotech	86	25	7	2	16
Eurocell	86	8	6	1	1
Fortress	86	29	8	4	17
Tulip	86	14	0	1	13

Only 1% (1/86) patient turned positive for brucellosis with all the tests kits and with both antigens (*B.abortus* and *B. mellitensis*) for the agglutination tests, except for Competitive Elisa (Table 4)

Forty patients (46.5%, 40/86,) were positive for brucellosis with either of the agglutination tests with at least one of the antigens (either *B. abortus*.or *B. mellitensis*).

Five patients (5.8. %; 5/86) tested positive for *Brucella abortus* in all the four agglutination tests, while 9% (8/86) of samples tested positive for *B. melitensis* alone.

(Table 4)

#### 4.3 Sensitivity and specificity of the different tests kits

Our gold standard which is competitive ELISA did not pick any positive sample; this does not rule out the possibility of there being brucellosis among our study patients because no culture for the isolation of brucella organisms was done. Therefore the sensitivity of the different agglutination kits is only zero as compared ELISA given the sample used in this study. The situation would have been different with a different set of samples with a known brucellosis status.

**Table 5: Sensitivity and specificity of the agglutination test kits according to this study**

Test	sensitivity	Specificity
Biotec	0	85
Fotress	0	71
Eurocell	0	66
Rose Bengal plate test	0	99.9
Tulip	0	91

#### 4.4 Comparison of Rose Bengal Plate Test kit with other test kits

Table 6 shows the summary of comparison between the different outcomes as depicted by use of different test kits. Only one patient tested positive on Rose Bengal plate test; and the same patient tested positive for brucellosis with the other four agglutination test kits. Eight patients tested positive on Eurocell. Of these; one was positive on Rose Bengal test, seven were positive on Biotech, six were positive on tulip and all were positive on fortress.

**Table 6: Agreement on the positive outcomes between Rose Bengal Plate Test and other agglutination Test Kits**

TEST	RB	Euro	Biotech	Tulip	Fortress
RB	1	1	1	1	1
Euro	1	8	7	6	8
Biotech	1	7	25	8	19
Tulip	1	6	8	14	9
Fortress	1	8	19	9	29

Twenty-five patients (29%) were positive for brucellosis when tested with Biotech test kit. Only one of them turned positive on Rose Bengal, seven of the twenty five were positive on Eurocell. eight of them turned positive on Tulip and nineteen of them turned out positive on Fortress test kit.

Thirteen patients tested positive for Brucellosis when tested with Tulip diagnostic test kit. Of these, only one was positive when tested with Rose Bengal test kit, six turned positive on Eurocell, and nine of them were still positive on Fortress test kit.

Fortress Brucellosis Test Kit was able to pick out twenty-nine patients as being positive for brucellosis. Of these, one tested positive on Rose Bengal, eight turned out positive on Eurocell, nineteen were positive on Biotech, while nine of them were positive on Tulip diagnostic Kit

The five samples that tested positive on all the agglutination test Kits, showed different reactions with the *B. abortus* and *B. melitensis* as shown in Table 7 below. The patients were all females. The symptoms associated with the five test positive patients were headache (3), fever (1), Joint pain (1), general malaise (4), backache (2), chills (1), and dizziness (1),

**Table 7: The distribution of *B. abortus* and *B. melitensis* in 5 samples testing positive on all commercial kits.**

Test kit	<i>B. abortus</i>				<i>B. melitensis</i>			
	Positive		Negative		Positive		Negative	
	number	%	number	%	number	%	number	%
Biotech	5	5.8	0	0	4	4.7	1	1.2
Eurocell	5	5.8	0	0	1	1.2	4	4.7
Fortress	5	5.8	0	0	4	4.7	1	1.2
Tulip	5	5.8	0	0	5	5.8	0	0



## CHAPTER FIVE

### 5.0 Discussion and conclusions

#### 5.1 Prevalence of Brucellosis

The prevalence of brucellosis in this community at the time of the project was dependent on the kit used to test the patient. Since Competitive Elisa was the gold standard, one can deduce from these results that there was no agreement between competitive ELISA and Rose Bengal plate test because as much as no patient turned out positive on ELISA, Rose Bengal plate test picked out one patient to be positive for brucellosis and the same patient was positive on all the other kits used.

With these results above, it is very worrying to note that all those patients who tested positive for brucellosis on the other locally available kits were treated for brucellosis when actually they did not have brucellosis according to Rose Bengal plate test except for one patient.

#### 5.2 Prevalence of Malaria and Typhoid

Malaria and typhoid were not a major problem to the study patients at the time of sampling since only one out of 105 patients was declared to have malaria and other two different patients tested positive for typhoid.

The thick blood film may also be used to make a limited haematological assessment because field stain preserves white blood cell morphology. As a result, it was possible to estimate the differential white blood count and to recognize nucleated red blood cells (which were not lysed). Reticulin (inside reticulocytes) stained blue. In *P. vivax* and *P. ovale* infections, parasites may be scanty in peripheral blood. In suspected infections several thick and thin blood films were made and examined carefully.

Therefore at the time of the study the two disease conditions were of very low prevalence, which is 0.95% for malaria and 1.9 % for Typhoid.

The purpose of culturing blood is to recover bacteria causing enteric fever septicaemia, endocarditis and secondary bacteraemia by culture of blood sample in broth culture then subculture on media of choice for further identification and performing susceptibility test of the isolated bacteria. In healthy subjects, blood is sterile. Blood cultures should also be done for diagnosis of localized infections (pneumonia, intra – abdominal abscesses, pyelonephritis, and meningitis)

### **5.3 Sensitivity of the tests**

From a practical perspective, to confirm a disease requires that you use a test with a high sensitivity because there are few false negatives. While to rule out a disease, you use a test with a high specificity because there are few false positives. In this study, the agglutination tests used had zero sensitivity but a high specificity therefore would not pass as being the best to be used for screening patients for the presence of brucellosis.

It is difficult to establish the correct incidence of diseases with non-specific symptoms as they are often misdiagnosed, and in cases where diagnostic tests are done, the prevalence of such a disease is likely to be higher than the incidence (Stollerman, 1975). The prevalence reported here is expected to be below the actual picture in Kibera (Table 1). Using competitive ELISA, the study patients from Kibera would have been declared Brucellosis free. Based on the Rose Bengal Plate test, the prevalence rate of 1.2% Biotec recorded 29.1%, Eurocell 9.3%, Tulip 16.3 %, and Fortress 33.7% .

Symptoms alone because of their unspecific nature are weak predictors of the likely diagnosis of brucellosis. Specific laboratory tests should be used to confirm clinical diagnosis as they have a better diagnostic power. This would be as follows; Blood culture for typhoid, Competitive Eliza for brucellosis and blood smear for malaria.

A big disparity exist between Rose Bengal plate test results and those of the other locally available test kits which are cheaper and readily available and therefore it would be better to use Rose Bengal Plate Test for screening patients suspected to suffer from brucellosis.

Before incurring huge expenses in treating unconfirmed disease status, health providers should consider how much would be saved if the proper and most reliable laboratory diagnostic procedures were done.

The results from this study indicate no brucellosis in the patient population studied. However, some of the test kits used to detect *Brucella* antibodies did not agree well, and therefore there is a need to re-evaluate the diagnostic tests for brucellosis before their use in health facilities.

Forty (40) positive samples by rapid test kits did not test positive on Competitive ELISA but these patients having been misdiagnosed using the rapid kits still had to go through therapy for brucellosis. In this Study, there was a strikingly high rate of symptoms headache, joint pains, general body weakness and abdominal discomfort. These results show that it is difficult to differentiate any of these diseases based on clinical signs. If a patient is free from the three diseases, other diseases that cause flu-like symptoms streptococcal infections and rheumatoid arthritis, should be considered or diseases whose antigens cross-reacts with those of brucella antibodies like *E. coli* O157; H7 and *Yersinia enterocolitica* 0:9.

In this study, it emerged that most patients who reported to have had joint pains, fever and headache were treated for Malaria, whereas any one presenting with abdominal discomfort, general malaise and headache was treated for typhoid fever even before the results of the blood culture was ready.

The probability of brucellosis based on clinical examination as per the findings of this study was too high for clinician to rule in a flu-like patient as having brucellosis. Given that all the patients that were registered for the study were suspected to have brucellosis, and subjecting such patients to a rose Bengal plate test would allow for a more definite decision

#### **5.4 Conclusion**

Clinical symptoms perceived by the patient and signs noted by the clinician are the basis of any diagnosis, either clinically or for referral using laboratory tests. Therefore, accuracy of clinical examination is crucial because it serves as the basis for most of the judgements about diagnosis, prognosis and therapy (Sacket et al., 1985). In previous studies, flu-like symptoms have been shown to be unspecific hence not useful in ruling in disease (Kengeya et al., 1994; Muriuki, 1994; Paul et al., 1995; Svenson et al., 1995). However, cultural examinations are time-consuming, hazardous and not sensitive. Thus, clinicians often rely on the indirect proof of infection. The detection of high or rising titers of specific antibodies in the serum allows a tentative diagnosis. A variety of serological tests has been applied, but at least two serological tests have to be combined to avoid false negative results. Usually, the serum agglutination test is used for a first screening and complement fixation or Coombs' test will confirm its results. As *Brucella*

ELISAs are more sensitive and specific than other serological tests, they may replace them step by step (Al Dahouk et al 2003).

Rapid and reliable, sensitive and specific, easy to perform and automated detection systems for *Brucella* spp. are urgently needed to allow early diagnosis and adequate antibiotic therapy in time to decrease morbidity / mortality and avoid misdiagnosis. The history of travel to endemic countries along with exposure to animals and exotic foods are usually critical to making the clinical diagnosis. Laboratory testing is indispensable for diagnosis. Therefore alertness of clinician and close collaboration with microbiologist are essential even in endemic areas to correctly diagnose and treat this protean human infection.

Existing treatment options, largely based on experience gained > 30 years ago, are adequate but not optimal. An initial combination therapy with a three drug-regimen followed by a two-drug regimen for at least six weeks and a combination of two drugs with a minimum of six weeks seems warranted to improve outcome in children and adult patients respectively with laboratory monitoring. A safe and effective vaccine in humans is not yet available. Prevention is dependent upon the control of the disease in animal hosts, effective heat treatment of dairy produce and hygienic precautions to prevent occupational exposure (Mantur et al 2007).

This study has shown that laboratory testing of patients with a high index of suspicion of any fever syndrome increases the probability of a correct diagnosis, hence improved diagnostic power. Malaria is undoubtedly well known. What is needed is to increase the awareness of health workers, especially clinical officers and the general population about

the existence of other conditions that manifest with similar symptoms, and soon they will realise the value of better diagnostic methods.

## **5.5 Recommendations**

These commercial rapid kits should be thoroughly evaluated before being used for the diagnosis of brucellosis in a hospital set up.

Rose Bengal plate test is a more sensitive test for diagnosis of brucellosis than the tested commercial rapid test kits.

Competitive ELISA is the most sensitive for the diagnosis of brucellosis.

## CHAPTER SIX

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## 7.0 APPENDICES


### 7.1 Ethical Clearance

#### Annex IV: Ethical clearance

**NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY**

Telegrams "SCIENCE TECH", Nairobi  
Telephone: Nairobi, 338173-5, 219420  
Fax: 333747, 212215-336176

When replying please quote  
Ref.No \_\_\_\_\_ and date \_\_\_\_\_



P. O. Box 30623  
NAIROBI  
.....20.....


NCST/5/C/486 2<sup>nd</sup> November, 2005

Dr. J. K. Wabacha  
University of Nairobi  
Department of Veterinary Medicine  
P.O. Box 29053  
Nairobi

**Re: Human Brucellosis in Kibera Nairobi**

Please refer to your application to conduct a study on human brucellosis in Kibera clinics in Nairobi. Following our verbal discussions which you addressed as per your letter of 7<sup>th</sup> October, 2005, you are hereby authorized to carry out the study for a period of two years starting December, 2007.

Please comply with the requirement that you deposit two copies of your final report when you complete the work.

  
R. N. Oduwo  
For : Secretary  
**NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY**

## Annex III: Research Authorization

### MINISTRY OF EDUCATION, SCIENCE & TECHNOLOGY

Telegrams: EDUCATION\*, Nairobi

Fax No.  
Telephone: 318541  
When replying, please quote



JOGOO HOUSE  
HARAMBEE AVENUE  
P. O. Box 310040  
NAIROBI  
KENYA

MOEST 13/228/2

5<sup>th</sup> October, 2005

**Dr. James H. Wabacha**  
**University of Nairobi**  
**Faculty of Veterinary Medicine**  
**Department of Clinical Studies**  
**P.O. BOX 29053**  
**NAIROBI**

Dear Sir

#### **RE: RESEARCH AUTHORIZATION**

Please refer to your application for authority to carry out research on "The prevalence of Human *Brucella* among patients attending health facilities in Nairobi with special emphasis in Kibera"

This is to inform you that you and your research team namely:

1. Dr. James H. Wabacha - Project Leader
2. Prof. Erastus Kimani Kang'ethe
3. Dr. Dennis Ongore
4. Prof. Njenga Muenesi
5. Dr. Otho Anzala
6. Dr. Andrew Juma Suleh

Have been authorized to carry out research in Kibera in Nairobi for a period ending 31<sup>st</sup> December, 2007.

You and your research team are advised to report to the Provincial Commissioner, the Provincial Director of Education, the Provincial Medical Officer of Health Nairobi and the District Officer, Langata Division before commencing your study.

Upon completion of the research, you are advised to submit two copies of your research report to this Office.

Yours faithfully

*M. O. Ondieki*  
**M. O. ONDIEKI**  
**FOR: PERMANENT SECRETARY**

Cc  
The Provincial Commissioner      The Provincial Director of Education  
Nairobi      Nairobi

The Provincial Medical Officer of Health  
Nairobi

The District Officer  
Langa's Division



PHD MOH R 1 VOL 1 (33)05

9<sup>th</sup> November, 2005

Dr. James Kihang'a Wabochi,  
University of Nairobi,  
College of Agriculture and Veterinary Science,  
Dept. of Clinical Studies,  
P.O. Box 29053,  
**NAIROBI.**

**RE: AUTHORITY TO CARRY OUT RESEARCH**

Reference is made to your letter dated 26<sup>th</sup> May, 2005 on the above subject matter.

I am pleased to inform you that permission has been granted for you to carry out research entitled "The Prevalence of Human Brucellosis among patients attending health facilities in Nairobi".

Please note that your study shall be restricted to only Kibera area of Langata District. This is however, subject to a payment of KShs.1200 = (One thousand two hundred only) Research fee.

By a copy of this letter the Assistant Medical Officer of Health – Langata District is requested to accord you the necessary assistance.

**MEDICAL OFFICER OF HEALTH  
NAIROBI CITY COUNCIL**

**DR. L.I. MUNENE  
FOR: MEDICAL OFFICER OF HEALTH**

✓ CC.  
AMOH - LANGATA DISTRICT

## 7.2 Form 1 A Patient Consent Form

**Project Title: Prevalence of human brucellosis among patients attending health facilities in Nairobi with special emphasis on Kibera residents**

This study is being conducted by the University of Nairobi, the Ministry of Health, and the African Medical and Research Foundation (AMREF) under the direction of Dr James Wabacha and Dr Jane Carter. The main purpose of the study is to identify individuals who are suffering from a disease called Brucellosis (ugonjwa wa maziwa), which is an infection acquired from domestic animals such as cows, goats and sheep, and to make sure patients receive correct treatment. Brucellosis may cause fever, joint pains, cough and other ailments that can be confused with malaria and typhoid. Therefore you will also be checked for malaria and typhoid, and will receive the correct treatment for these conditions, if present.

20 ml of blood will be taken from one of your veins and will be used only for the purpose of this study. The blood will be tested for malaria, typhoid and brucellosis only. The results will be kept confidential. If any of these diseases are found to be present, you will receive the correct treatment.

I understand that I may refuse consent to participate in the study without any penalty or loss of benefit, and that in this case I will receive the usual investigations and treatment provided by the health facility.

**Declaration:**

I (or parent/guardian for a patient less than 18 years) declare that the project purpose has been fully explained to me, that I have been given an opportunity to ask questions, and that all my questions have been answered satisfactorily. I consent to having my blood drawn for the intended tests only.

Name .....

Identification Card Number .....

Study Number ..... Telephone number .....

Signature ..... Date .....

Witness name ..... Witness designation .....

Witness signature ..... Date .....

**7.3 Form 1B Clinical Data Capture Form**

**Project Title: Prevalence of human brucellosis among patients attending health facilities in Nairobi with special emphasis on Kibera residents**

<b>PATIENT'S DETAILS</b>	
Patient's names: 3 names and commonly known names	
Telephone No.	
Clinic Registration No.	
Date of birth/age	(1) Date of birth (2) Age
Sex	(1) Male (2) Female
If Female	Date of LMP
Residence (give details)	If from Kibera: (1) Makina (2) Mashimoni (3) Kisumu Ndogo (4) Silanga (5) Lindi (6) Gatwikira (7) Soweto (8) Laini Saba Other areas (Specify):
<b>PATIENT'S HISTORY &amp; EXAMINATION (select more than one answer if required)</b>	
Travel history - in last 60 days	(1) Stayed in Nairobi: (2) Travelled to rural (state where):
Animal contact – in last 60 days	(1) Cows: (6) Camels (2) Sheep: (7) Others (specify): (3) Goats: (4) Pigs: (5) Dogs
Occupation/activities – in last 60 days	(1) Milk hawker or seller (2) Butcher (3) Abattoir worker (4) Livestock keeper/herder (5) Animal health service provider (6) Meat inspector (7) Preparing/selling hides & skins (8) Others (specify):
Source of milk or milk products: cheese, butter, maziwa lala, etc	(1) Own livestock (2) From a hawker (3) From neighbours' livestock (4) From a shop/kiosk (5) Others:

Type of milk purchased	(1) Commercial packet (2) Raw milk (3) Others:
Treatment of milk	(1) Taken raw (2) Boiled (3) Soured (lala) (4) Others:
How often milk products are consumed	(1) Daily: (2) Weekly: (3) Others:
Clinical symptoms	(1) Fever (2) Chills (3) Night sweats (4) Joint pain/arthritis (5) Back pain (6) Cough (7) Headache (8) Depression (9) Others:
Duration of symptoms	(1) Days. (2) Weeks. (3) Months.
Previous treatment for same/similar condition	(1) When: (2) Where: (3) Type of treatment/drugs used:
Signs	(1) Temperature ..... (2) Lymphadenopathy (3) Enlarged liver (4) Enlarged spleen (5) Peripheral arthritis (6) Others:
<b>LABORATORY INVESTIGATIONS</b>	
Blood slide for malaria	(1) Positive (2) Parasite count /200 WBC (3) Species (4) Negative

**FORM 1B**

**STUDY NO:** \_\_\_\_\_

Rose Bengal test	(1) Positive (2) Negative
Usual <i>Brucella</i> test performed at the facility	(1) Positive (2) Titre .... (3) Negative
Competitive ELISA for <i>Brucella</i>	(1) Positive (2) Negative
Blood culture for <i>Salmonella</i>	(1) Positive (2) Organism (3) Negative
Others	
<b>DIAGNOSIS</b>	(1) Brucellosis: (2) Malaria: (3) Typhoid: (4) Others:
<b>TREATMENT GIVEN</b>	
Name of drug(s), dose & duration	(1) Streptomycin 15 mg/kg IM x 3 weeks (2) Doxycycline 100 mg bid Po x 6 weeks (3) Others specify.....
Other management	
Follow up advice	
Other comments	
<b>TO BE COMPLETED BY CLINICIAN</b>	
Name of clinician	
Signature	
Date	

Notes: Write the study number on each sheet.

**7.4 Form 1C Laboratory Request Form**

**Project Title: Prevalence of human brucellosis among patients attending health facilities in Nairobi with special emphasis on Kibera residents**

Patient's names (2 – 3 names)	
Age	
Sex	
Clinic registration no	
Clinical summary	
<b>LABORATORY INVESTIGATIONS REQUESTED</b>	
Blood slide for malaria	
Rose Bengal test	
Usual <i>Brucella</i> test performed at the facility	
Competitive ELISA for <i>Brucella</i>	
Blood culture for <i>Salmonella</i>	
Others	
<b>ORDERED BY</b>	
Name of clinician	
Signature	
Date	

(On reverse of form)

<b>LABORATORY RESULTS</b>	
Blood slide for malaria	(1) Positive (2) Parasite count                      /200 WBC (3) Species (4) Negative
Rose Bengal test	(1) Positive (2) Negative
Usual <i>Brucella</i> test performed in the facility Name _____	(1) Positive (2) Titre. (3) Negative
Others	
<b>EXAMINED BY</b>	
Name of technologist	
Signature	
Date	

Notes: Write the study number on each sheet.

## 7.5 Form 1D Specimen Referral Form

STUDY NUMBER \_\_\_\_\_

Date submitted \_\_\_\_\_

(Do NOT fill in the Laboratory results at the facility).

**Project Title: Prevalence of human brucellosis among patients attending health facilities in Nairobi with special emphasis on Kibera residents**

Patient's names (2 – 3 names)	
Laboratory number	
<b>LABORATORY INVESTIGATIONS</b>	
Blood slide for malaria	(1) Positive (2) Parasite count /200 WBC (3) Species (4) Negative
Rose Bengal test	(1) Positive (2) Negative
Usual <i>Brucella</i> test performed at the C. Laboratory Name	(1) Positive (2) Titre .... (3) Negative
Competitive ELISA for <i>Brucella</i>	(1) Positive (2) Negative
Blood culture for <i>Salmonella</i>	(1) Positive (2) Organism (3) Negative
Others	
<b>TO BE COMPLETED BY LABORATORY TECHNOLOGIST</b>	
Name of technologist	
Signature	
Date	



## 7.6 Form 1 E Clinical Follow-Up Form

**Project Title: Prevalence of human brucellosis among patients attending health facilities in Nairobi with special emphasis on Kibera residents**

<b>PATIENT'S DETAILS</b>	
Patient's names (2 - 3 names and commonly known name)	
Telephone number	
Clinic Registration No.	
Date of birth/age	(1) Date of birth (2) Age
Sex	(1) Male (2) Female
If Female	Date of LMP
Residence (give details)	If from Kibera: (1) Makina (2) Mashimoni (3) Kisumu Ndogo (4) Silanga (5) Lindi (6) Gatwikira (7) Soweto (8) Laini Saba Other areas (Specify):
<b>PATIENT'S HISTORY &amp; EXAMINATION</b>	
Reason for return	
Clinical symptoms	(1) Fever (2) Chills (3) Night sweats (4) Joint pain/arthritis (5) Back pain (6) Cough (7) Headache (8) Depression (9) Others:
Duration of symptoms	(1) Days ..... (2) Weeks ..... (3) Months.....
Treatment prescribed & taken	(1) Streptomycin 15 mg/kg IM x 3 weeks (2) Doxycycline 100 mg bd Po x 6 weeks (3) Others specify.....
Other treatment sought & taken	

**STUDY NO:** \_\_\_\_\_

Signs	(1) Temperature ..... (2) Lymphadenopathy (3) Enlarged liver (4) Enlarged spleen (5) Peripheral arthritis (6) Others:
<b>FURTHER LABORATORY INVESTIGATIONS</b>	
Blood slide for malaria	(1) Positive (2) Parasite count ...../200 WBC (3) Species (4) Negative
Blood culture for <i>Salmonella</i>	(1) Positive (2) Organism (3) Negative
Others	
<b>DIAGNOSIS</b>	(1) Brucellosis: (2) Malaria: (3) Typhoid: (4) Others:
<b>TREATMENT GIVEN</b>	
Name of drug(s), dose & duration	(1)..... (2)..... (3).....
Other management	
Follow up advice	
Other comments	
<b>TO BE COMPLETED BY CLINICIAN</b>	
Name of clinician	
Signature	
Date	

Notes: Write the study number on each sheet.

## 7.7: Treatment Regimes Given to Some Study Patients.

Patient no.	Clinical signs	Treatment	Laboratory results		
			Brucellosis	Malaria	Typhoid
K002	Fever, Abdominal discomfort. General malaise	Tinidazole, Brufen	-	-	-
K003	Fever, chills, headache, Abdominal	Tinidazole, Brufen	-	-	-
K004	Joint pain, backache, General malaise	No treatment	-	-	-
K007	Joint pain, Abdominal distension, Backache	Brufen, Multivitamin, antacid	-	-	-
K008	Cough, fever, chills, headache, joint pain	Cetamol, multivitamin	+	-	-
K009	Fever, joint, cough, backache	Normax, Brufen, flagyl	-	-	-
K010	Stomach ache, fever, joint pain	Normax, flagyl, cetamol	-	-	-
K013	Fever, chills, headache, backache, abdominal pain	Septin, multivitamin, diclophenac	+	-	-
K016	General malaise	Buscopan	-	-	-
K017	Headache, joint pain, cough, chest pain backache.	Amobin, cetamol	+	-	-
K018	Neck pain, joint pain, dizziness, cough, fever	Septin, flagyl, buscopan	-	-	-
K019	Headache, backache, lumber pain	Brufen	-	-	-
K021	Joint pain, backache, chest pain, fever	Brufen, diclophenac	-	-	-
K022	Dizziness, headache, backache	No treatment	+	-	-
K023	General malaise, backache	Cetamol, antacid flagyl	+	-	-
K026	General malaise	No treatment	+	-	-
K027	Joint pain, headache, prolonged fever	Amobin, cetamol,	-	-	-
K028	Sever joint pain,	Diclophenac ,Amobin	+	-	-

Patient no.	Clinical signs	Treatment	Laboratory results		
			Brucellosis	malaria	Typhoid
K030	Fever, backache, abdominal pain, joint pain	Brufen. Flagyl	-	-	-
K035	Chills, joint pain, headaches,	Amobin, cetamol	-	-	-
K036	Joint pain, backache, fever,	Brufen, antacid, multivitamin	-	-	-
K037	Nausea, dizziness, headache, Backache	Amobin, cetamol, multivitamin	-	-	-
K039	Headache, fever, abdominal discomfort	Doxycycline, septrin, folic acid, buscopan, cetamol	-	-	-
K041	Headache, General malaise	Doxycycline, septrin, folic, buscopan, cetamol	+	-	-
K042	Headache, General malaise	Doxycycline, Septrin, folic acid, Buscopan, cetamol	+	-	-
K043	Chest pain, stomach ache, headache	Doxycycline, septrin, folic acid, buscopan, cetamol	+	-	-
K046	Headache joint pain, general malaise stomach discomfort.	Cetamol, antacid	+	-	-
K047	Headache, fever, chest pains	Cetamol, antihistamin	-	-	-
K050	Hip joint pain, headache, backache, abdominal pain	Indocid	-	-	-
K051	Fever, Chest pain, Backache	Diclophenac	+	-	-
K052	Chills, fever, headache, joint pain	Cetamol, piriton, septrin.	+	-	-

		.doxycycline			
K054	Fever, backache, headache	Amobin, Brufen, sp tabs	-	-	-
K055	General Malaise	Normax, Fansider, Amobin	-	-	-
K056	General malaise	Brufen, Erythromycin	-	-	-
K064	Palpitations, fever, general malaise	Doxycycline, septrin, Buscopan	+	-	-
K067	Cough, chest pain, headache, backache, joint pain	Amobin, Brufen, Multivits	+	-	-
K070	Chills, Headache Backache	Flagyl, Brufen, multivits	-	-	-
K075	Abdominal pain, headache for several months	Amoxil, vit c Brufen,	-	-	-
K077	Joint pain, fever, Chills	Septrin, Doxycycline, Brufen	-	-	-
K079	Fever, chills, headache, abdominal pain, joint pain	Tinidazole, normax, Levamizole, Alogel, paracetamol, diazepam	-	-	-
K081	Chills, joint pain, general malaise	Tinidazole, brufen, Vitamin c, alogel.	-	-	-
K084	Cough chest pain, headache, neck pain, chills.	Cough syrup, Brufen, Amoxil.	-	-	-
M001	Fever, chills, Headache	Brufen, cough syrup	+	-	-
M002	Headache, Stomach ache, General malaise	Diclophenac, Flagyl	-	-	+
M003	General malaise, Chills, in appetence	Brufen, multivitamin	-	-	-
M004	Fever, Backache, Abdominal discomfort.	Tinidazole, Brufen, multivitamin	+	-	-

## 7.8: Stains and Solutions Used for Blood Smears

Field Stain consists of two solutions:

### Field's A:

Methylene blue	0.8g
Azure I	0.5g
Disodium hydrogen Phosphate (anhydrous)	5.0g
Potassium Dihydrogen Phosphate (anhydrous)	6.25g
Distilled water	500ml

### Field's B:

Eosin	1.0g
Disodium Hydrogen Phosphate (anhydrous)	5.0g
Potassium dihydrogen Phosphate (anhydrous)	6.25g
Distilled water	500ml

The salts are first dissolved, the stain is added and solution of azure I aided by grinding in a mortar. The stain is set aside to for 24 hours, filtered and the used. The Prepared film is dipped into solution A for 1 second, gently rinsed in clean water for a few seconds till stains Ceases to flow from the film, dipped for 1 second into solution B, gently rinsed for 2 to 3 seconds and placed vertically in a rack to dry.

## 7.9 Gram Staining Procedure for *Salmonella typhi*.

Blood culture bottles are gently mixed and 0.2 ml of the broth is drawn with a sterile needle and syringe. A glass slide is then cleaned with gauze and labelled with a laboratory number with a grease pencil. A smear is then made of the broth on a glass slide and let to dry. The smear is then fixed with absolute alcohol and let to dry.

The smear is then stained with 0.5% Gentian Violet for one minute, excess stain poured and slide washed gently with tap water. Grams Iodine is added, left to stand for one minute then washed with water. The slide is decolourised with 50% acetone/alcohol until no blue colour flows out then washed with water.

The smear is then counter stained with dilute carbolfuchsin for one minute and washed with water then let to dry. The slide is then placed on a microscope stage, examined with x10 objective to get the focus of the material then x100 oil immersion objective for the microorganisms.

