A STUDY ON BRUCELLOSIS IN KOSTI AREA, WHITE NILE STATE, SUDAN

By

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DEDICATION

This work was dedicated to the

soul of My father

My mother,

Brothers and sisters

PREFACE

This work has been carried out at the Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum, Under supervision of Dr. Isam Mohammed Ali Eljalii

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ABSTRACT

This study was conducted in Kosti area in order to investigate bovine and human brucellosis. 113 milk samples and 120 serum samples were collected from bovine dairy farms, while 100 serum samples were collected from human presented to Kosti Hospital with different clinical manifestations. All serum samples were examined by rose Bengal plate test ((RBPT) to demonstrate antibodies to brucella while all milk samples were examined by milk ring test (MRT) and modified Zeihl Neelsen stain (MZN). The results showed that the prevalence of human brucellosis was 2% based on rose Bengal plate test (RBPT), while the prevalence of bovine brucellosis in selected dairy farms was 12.39% based on modified Zeihl Neelsen stain (MZN).

Sensitivity and specificity of the tests used in this study were calculated to evaluate these tests for detection of brucellosis. The sensitivity and specificity of milk ring test (MRT) were very high (100% and 95.96%, respectively). While (RBPT) showed high specificity (95.96%) and low sensitivity (50%).

Application of agreement between tests (Kappastatistis) showed perfect agreement (0.85) between MZN and MRT. While moderate agreement was observed for MZN and RBPT , RBPT and MRT (0.50 and 0.56, respectively).

The results of risk factor analysis revealed that number of calves as well as hygiene status in diary farms were not associated with occurance of bovine brucellosis (X^2=3.98 and 0.69, respectively , P>0.05).

In contrast, there was a positive correlation between the presence of bovine brucellosis and history of abortion (X^2=33.55, P<0.01). The Odds Ratio (OR=32) confirmed that the history of abortion, could be risk factor for occurance of bovine brucellosis. It could be concluded that bovine and human brucellosis are found in Kosti area .

ملخص الاطروحه

أجريت هذه الدراسة بمدينة كوستي بغرض تقصي حدوث الإجهاض المعدي في الأبقار والحمي المالطية في الإنسان. تم أخذ 113 عينة لبن و 120 عينة دم من مزارع ابقار وأيضا تم أخذ 100 عينة دم من مرضي في مستشفي كوستي احضروا بعلامات سريريه محتلفه كل عينات المصل فحصت بواسطة الروز بنقال لكشف الاجسام المضاده لمرض الاجهاض المعدي بينما فحصت كل عينات اللبن باختبار اللبن الحلقي واختبار صبغة زلنسون. أظهرت النتائج بأن نسبة حدوث المرض في الإنسان كانت 2% من 100 عينة دم بواسطة اختبار الروز بنقال بينما نسبة حدوث المرض في الأبقار كانت 12.39% من 113 عينة لبن بواسطة اختبار صبغة زلنلسون.

تقييم بعض الاختبارات السيرولوجية المستخدمة في تشخيص مرض الإجهاض المعدي في الأبقار أظهرت بأن اختبار الحساسيه والنوعية لاختبار اللبن الحلقي كان نسبياً مرتفع (100% و 95.96% علي التوالي). بينما أظهر إختبار النوعية بالنسبه لاختبار الروز بنقال ارتفاعاً (95.96%)، بينما انخفض اختبار الحساسيه لاختبار الروز بنقال الي 50%.

تطبيق اختبار التطابق بين الاختبارات المختلفه لتشخيص مرض الإجهاض المعدي في الأبقار. اظهر تطابق عالي بين اختبار صبغة زلنلسون واختبار اللبن الحلقي (0.85) بينما التطابق كان متوسطاً بين اختباري صبغة زلنلسون والروز بنقال وايضاً بين اختباري الروز بنقال واللبن الحلقي (0.50 و 0.56 علي التوالي).

نتائج تحليل العوامل الخطرة أظهرت ليست ثمة علاقة بين عدد الولدات والوضع الصحي في المزرعة وحدوث مرض الإجهاض المعدي في الأبقار (اختبار مربع كاي= 3.98 و 0.69 علي التوالي وقيمة P

اكبر 0.05) . علي العكس وجدت ان هناك علاقة وثيقة بين حدوث المرض وتاريخ حالات الإجهاض (اختبار مربع كاي 33،55 وقيمة P اقل 01،) وتم تأكيد النتيجة بواسطة اختبار النسبة الشاذة (32) حيث أظهرت بأن تاريخ حالات الإجهاض يعتبر عامل خطوره بالنسبه لحدوث المرض خلصت الدراسة ان المرض موجود في منطقة كوستي.

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INTRODUCTION

Brucellosis is a zoonotic disease that causes great losses to animal production and affects public health causing undulant fever or human brucellosis. The diseases is caused by members of the genus Brucella.

Brucella has six species that are recognized, *Br.abortus* (cattle); *Br.melietnsis* (sheep and goats); *Br.suis* (swine); *Br.ovis* (sheep); *Br.canis* (dogs) and *Br. neotomae* (desert wood rat) (Bergy's, 1984). Brucellosis has been reported throughout the world since its discovery in the later part of the nineteenth century. The first isolation of Brucella organisms from animals was made by Bang (1897).

In the Sudan, the first isolation of *Br.abortus* was made by Bennet in 1943 from a Friesian herd at Bulgravia Dairy Farm. But the first isolation of *Br.abortus* from local cattle was from a cow which aborted at Juba Dairy Farm (Dafala , 1962).

Prior to the use of the name brucellosis, the disease in cattle was known by many names. Those were infectious abortion, which was also referred to as Bang's disease, slinking of the calve and contagious abortion. Bovine brucellosis was recognized as a contagious disease since 1878, when the disease was produced by transferring part of an infected placenta into the vagina of healthy pregnant cow. In man, the disease was also known as Malta fever, Mediterranean fever, undulant fever and goat fever (Carpenter and Hubbert, 1963).

The disease is transmitted by many ways mainly ingestion and is characterized by contagious abortion in animals and febrile illness in man (Radostits *et al.,* 2000). The

disease in cattle caused by *Br.abortus*. Regarding the pathogenicity in cattle, abortion is the most frequent observed symptom but other manifestions occur such as hygromas, orchitis, retention of placenta, weak or still births, long calving intervals, infertility, bursitis and arthritis (Mereck, 1998).

Diagnosis of brucellosis mainly depend on detection of bacteria using modified Ziehl Neelsen stain (MZN) and serological test such as milk ring test (MRT), rose Bengal plate test (RBPT), serum agglutination test (SAT), complement fixation test (CFT), and enzyme linked immunosorbent assay (ELISA). The confirmation of brucellosis will be done by using bacteriological isolation. Recently PCR has been developed for detection brucellosis in animals (Radostits *et al.,* 2000; Amel, 2005).

Brucellosis is a disease of both public health and economic importance and it is of world wide distribution. Losses of animal production due to brucellosis include diminution of milk and meat, abortion, infertility, long calving intervals and higher culling rates (Blood *et al.,* 1983).

Treatment of brucellosis is limited and the control can be achieved by reduce reservoir of infection , quarantine, depopulation, and vaccination (Radostits *et al;* 2000).

In Sudan, brucellosis was recognized in different species of animals such as cattle, sheep, goats, camels, and wild animals in different parts of the country. There is no survey concerning bovine and human brucellosis has been done in Kosti area. There fore the objectives of this study were:

1. To determine the occurrence of bovine and human brucellosis in Kosti area

2. To evaluate some serological tests, that are used for diagnosis of bovine brucellosis.
3. To find out the relationship between some factors and occurance of bovine brucellosis.

CHAPTER ONE

LITERATURE REVIEW

1.2 Brucellosis

1.1.1 Definition

Brucellosis is a serious zoonotic disease . It caused by any one of the members of the genus Brucella (Br). The causative bacterium was named in honour of Bruce (1887) the discoverer of *Brucella melitensis* . Prior to the use of the inclusive term brucellosis, the disease in cattle was known by many names. Those were infectious

abortion which was also referred to as bang's disease, contagious abortion and slinking of the calf. In man the disease was also known as malta fever, mediterranean fever, and goat fever which are often synonomously used for undulant fever (Carpenter and Hubbert, 1963).

1.1.2 Historical Background

The first isolation of the organism from spleen of human with Malta fever was by Bruce (1887) and named it *Micrococcus melitensis*., Mohler and Tram (1911) isolated *Brucella abortus* from a guinea pig inoculated with tonsil material from a child and that was the first instance in which the organism was isolated from a human source. Evans (1918) pointed out that *Micrococcus melitensis* described by Bruce and *Bacillus abortus* isolated by Bang were morphologically and antigenically similar. (Young and Corbel, 1989).

The first isolation of Brucella organisms from animals was made by Bang (1897), who was the first to report contagious abortion in cattle and other animals species and he named his isolate *Bacillus abortus* , which was followed by other names, *Corynebacterium abortus, Bacterium abortus* and *Alcaligenes abortus*. It is in recognition of Bang's work that brucellosis in cattle is often named Bang's disease. Meyer and Shaw, in 1920 suggested the name Brucella for the genus.

In Sudan, animal brucellosis was suspected as early as 1904. The first isolation of *Br.abortus* was made by Bennet in 1943 from a Friesian herd at Bulgravia Dairy Farm in Khartoum. Hasseb was confirmed the first case of human brucellosis (Hasseb,

1950). But the first isolation of *Br.abortus* from local cattle was from cow which aborted at Juba Dairy Farm (Daffalla, 1962).

1.3 Bovine Brucellosis

Bovine brucellosis is a highly contagious bacterial disease caused by genus Brucella. The dominant feature is late-term abortion and infertility in cattle. The disease is also a serious zoonosis, causing undulant fever in human. Cattle are the most important source of infection with *Br.abortus* but other bovidae can be of local importance (Casolinuovo *et al.*, 1996).

1.3.1 Etiology

Bovine brucellosis is caused by members of the genus Brucella which is a small Gram-negative, non-motile, non-capsular, coccobacillus or short rod, normally intracellular in host tissues. It is not acid fast and stains red with stamp's modification of the Ziehl-Neelsen stain. The cell varies from 0.4 to 1.5 um in length and 0.4 to 0.8 um in width. Young colonies are pinpoint in size, moist, translucent and glistering (Spink, 1986).

1.3.2 World Distribution

Bovine brucellosis was widely distributed throughout the world. A number of countries , including several in Europe and Scandinavia, Australia, New Zealand, Canada, Israel and Japan have succeeded in eradicating the disease (Ozekicit *et al*, 2003).

1.2.3 Transmission

Transmission of brucellosis is very likely to occur via the oral route because cattle tend to lick aborted fetuses and the genital discharge of an aborting cow (Cunningham, 1977). Exposure to Brucella organisms is also likely occur in uterus (Fensterbank, 1978) or when calves born to healthy dams are fed on colostrums or milk from infected dams (Bercovich *et al* ., 1990, Radostits *et al*., 2000).

It has been established that brucellosis in bulls does not always result in infertility, although semen quality may be affected. Bulls that remain fertile and functionally active will shed Brucella organisms with the semen during the acute phase of the disease. Shedding, however, may cease or become intermittent (Mccaughey *et al*., 1973). In contrast to artificial insemination, bulls used in natural service may fail to spread the infection, as the infected semen is not deposited in the uterus (Ray, 1979). Brucella infections are usually transmitted by oral exposure, but other routes also known such as inhalation, conjunctival exposure, direct skin contact (Nicoletti, 1980). Humans are infected through handling infected cows or their tissues, or through drinking infected milk or by inhalation, through conjunctiva and skin (Corbel, 1989a).

1.2.4 Pathogenesis

The organisms was first found by Bang (1897) in the uterochorionic space of aborted cow. Many of the organisms were enclosed in the protoplasm of epithelial cell (Hagn's 1973). Cunningham (1977) showed that these epithelial cell were derived from the outer foetal envelope and the chorion.

Spink (1986) reported that the three classic species of Brucella were highly invasasive gaining entrance through the oral or ocular mucosae or through abrasions of the skin

and it could invade the respiratory tract. A state of bacterial hypersensitivity similar to that in tuberculosis was induced. Yassin *et al*. (1987) reported that in the uterus of the pregnant cow there was rapid multiplication of bacteria during the second and third trimester of pregnancy. Both humoral and cellular mediated immunity have been shown to contribute to protective immunity against Brucella. Winter (1990) has reviewed studies on humoral and cell mediated immune response (CM) and suggested that protective immunity against *Br. abortus* in mice was due to combined effects of antibodies and of (M) responses mediated by T cells. Cunningham, (1977) observed that immunomdulators like bovine interferons could reverse suppression of neutrophils function by virulent brucella. *Br.abortus* might pentrate the mucosa of nasal, oral or pharyngeal cavities were then transported either free or within the phagocytic cell to regional lymph node, bacteraemia occurred with localization in other lymph tissues (Spleen, Iliac, mesenteric and supramammary lymph nodes). Hyperplasia at these sites lead to granulomata. The organism then spread via lymph and blood to other organs. (Quinn *et al*., 2002).

1.2.5 Epidemiology

Cows shed large numbers of organism into the environment when they abort. Cows that lactate following abortion excrete bacteria intermittently in milk throughout the lactation period. Urine faces and hygroma fluids are also a source of bacteria. There is a rapid decline of organisms soon after calving or abortion, and cows are then generally non-infective until the next pregnancy, when there again arapid build-up of Brucella organisms in the reproductive tract (even in absence of abortion). Most cows

remain chronically infected. With infection localizing in the udder and lymph nodes (Nicoletti, 1984).

The Brucella is intracellular parasite, so they have protection from the innate host detenses and from their therapeutics agents. Natural or artificial infection usually persis indefinitely although a bout 10-15% recover spontaneously (Nicoletti, 1980).

Environmental survival of the organism depends on temperature and exposure to sunlight. It may survive for up to eight months in an aborted foetus in the shade, for 3-4 months in faeces, and for 2-3 months in wet soil (Wary, 1975). Human infection is a equired either occupationally through handling infected cows or their tissues or discharges, or through drinking infected milk. The latter route of infection is prevented by pasteurization.

1.2.6 Incubation Period

The incubation period is variable and often can not be accurately determined. The length of incubation period was inversely proportional to the stage of foetal development at time of exposure (Thomsen, 1950). The incubation period in brucellosis is affected by several factors such as gestation, exposure, dose, age, vaccination and other unknown host resistant influences (Nicoletti, 1980).

1.2.7 Clinical Signs

Brucellosis affects many different organs in animals and consequently the signs of the disease will be influenced by the nature and extent of the infection and the species involved. Some infected animals may not show signs. (Bishop *et al;* 1994), the dominant feature of the disease in cattle is abortion. Usually occurs at about 5-7

months, full-term calves may die soon after birth. Abortion rates in herd vary. In fully susceptible herds rates may vary from 30% to 80%. Retained placenta and secondary metritis is common and may lead to permanent sterility. In bulls, acute or chronic infections of the reproductive tract may occur (orchitis, epididymitis, seminal vesiculitis, hygromas, particularly of the carpal joints, occur in some animals in chronically affected herds) (Bishop *et al.,* 1994).

1.2.8 Diagnosis

Diagnosis of *brucellosis*. can be done or carried out based on the:

a. Clinical signs
b. Laboratory

1.2.8.1 Clinical Signs

An out break of brucellosis is hardly ever confined to one animal and there are no pathognomonic signs. Therefore, clinical examination of aborted material is not of great diagnostic value. (Corbel, 1973).

1.2.8.2 Laboratory Diagnosis

The laboratory tests include isolation or demonstration of the organisms from tissues or fluids, serological tests, agglutination tests in milk or seminal plasma and molecular diagnosis

1.2.8.2.1 Culturing of Samples for Isolation

Culture of suitable material on one of the Brucella media and isolation of causative agent.

1.2.8.2.2 Demonstration of Brucella Organisms in Suspected Samples

By staining with either modified Koster's method (Christofferson and Ottosen, 1941) or modified Ziehl Neelsen's stain. These methods are not specific for brucella organisms, but *Coxiella Burnetti* was found to be stained red as Brucella (corbel, 1973).

1.2.8.2.3 Microscopical Identification by Immuno-fluorescens

This method was specific and dependable in differentiating between Brucella infection and that of Q-fever (Corbel, 1973).

1.2.8.2.4 Guinea Pig Inoculation:

This method is more successful than direct culture especially from contaminated material. Guinea pigs are injected intramuscularly and killed after 4-5 weeks of inoculation. Then their sera are tested by serum agglutination tests (SAT). Recovery of the organism from the spleens or positive serum agglutination test (SAT) at 1/10 serum dilution or over are taken as evidence of infection (Brinely *et al;* 1978).

1.2.8.2.5 Serological Tests:

Many serological tests were developed for diagnosis of brucellosis using body fluids such as sera, hygroma fluids, milk, vaginal mucus, semen, bursa and muscle juies from suspected cattle these fluids may contain different quantities of antibodies of the M, G_1, G2, and other types directed against Brucella (Beh, 1974). Because infected cattle may or may not produce all antibody types in detectable quantities several tests are used to detected brucellosis. The commonly used tests are the milk ring test (MRT), serum agglutination test (SAT), complement fixation test (CFT), rose Bangal

plate test (RBPT), anti-glubulin (Coombs) test, enzyme-linked immunosorbent assay (ELISA).

1.2.8.2.5.1 Milk Ring Test (MRT)

The milk ring test (MRT) is cheap, easy, simple and quick to perform. It detects lacteal anti-brucella Ig M and IgA bound to milk fat globules. However, it tests false positive when milk that contains colostrums, milk at the end of the lactation period, milk from cows suffering from a hormonal disorder or milk from cows with mastitis (Bercovich and Moerman, 1979). Milk that contains low concentrations of lacteal IgM and IgA or which is lacking the fat-clustering factors tests false-negative (Keer *et al*; 1959; Tanwani and Pathak, 1971; Patterson and Deyoe, 1978). Because lacteal antibodies rapidly decline after abortion or parturition, the reliability of the MRT, using 1 ml milk, to detect Brucella antibodies in individual cattle or in tank milk is strongly reduced (Hill, 1966). Although the MRT performed with 8 ml milk it improved the detection of brucellosis in tank milk (Bercovich and Lagendijk, 1978), it may be false positive when traces of colostrums are present in tank milk (Bercovich and Moerman, 1979). According to WHO report, (1992) the MRT is not suitable for sheep and goats as ring formation does not readily occur.

1.2.8.2.5.2 Serum Agglutination Test (SAT)

This test is widely used in some countries and its positive results are subjected to the definite CFT. Other than sera, the agglutination can be used for vaginal mucous and semen examination. The antigen used in the test is a Brucella whole cell and the antibodies detected are those directed against the surface molecules. SAT unlike the

other tests, it detects antibodies of other isotypes (MacMillan, 1990). It can be performed in tubes or microtitre plates and the plate was found to be more sensitive (Herr *et al.,* 1982). SAT has international standardization, it is used for control programmes and import and export policies (MacMillan and Cockrem, 1985). The two investigators also reported that, sometimes non-specific agglutination occurred in the test using known negative sera due to non-immune binding of bovine Ig M to cells of *Br.abortus.* Morgan *et al*., (1969) mentioned that a proportion of sheep bacteriologically positive for brucellosis failed to react to the SAT. This proved the inferiority of SAT compared to the other conventional test. According to reports of FAO/WHO Expert Committee on Brucellosis (1964), the results of this test in cattle with antibody level less than 50 I.U should be considered negative in non-vaccinated animals or in those with unknown vaccination history. Whereas in the vaccinated over 30 months of age, the level should be more than 50 I.V. In camels the level of a positive titre has been established. (ElNahas, 1964) considered a titre of 1/40 i.e. (50% agglutination at 1/40 serum dilution) and above as positive SAT is modified by addition of 10% sodium chloride to diluent and this is found to abrogate prozone phenomenon which is due to high concentration of IgG_1 (Kolar, 1989). Falada, (1978), compared RBPT, SAT and MRT for diagnosis of brucellosis in caprine and concluded that SAT offered a better serological result.

1.2.8.2.5.3 Complement Fixation Test (CFT)

This test is used for confirming the result of the RBPT and SAT. The test was found to be more accurate for bovine brucellosis (Morgan *et al*., 1973). The

complement fixation test (CFT) detects specific antibodies of the Ig M and Ig G types that fix complement (Hill, 1963, Levieux, 1974). The CFT is highly specific (Hill, 1963). But it is laborious and requires highly trained personnel as well as suitable laboratory facilities. This makes the CFT less suitable for use in developing countries. Although its specificity is very important for control and eradiation of brucellosis it may test false negative when antibodies of the Ig G_2 type hinder complement fixation (MacMillan, 1990). The CFT measures more antibodies of the Ig G_1 type than antibodies of the Ig M type, as the latter are partially destroyed during inactivation. Since antibodies of the Ig G 1 type usually appear after antibodies of the IgM type control and surveillance for brucellosis is best done with SAT and CFT (Levieux, 1974).

1.2.8.2.5.4 Rose Bengal Plate Test (RBPT)

The rose bengal plate test is a spot agglutination technique. Because the test does not need special laboratory facilities and it is simple and easy to perform, it is used to screen sera for Brucella antibodies. The test detects specific antibodies of the Ig M and IgG types and is more effective in detecting antibodies of the Ig G_1 type than Ig M and Ig G_2 types (Levieux, 1974). The test may yield negative result in infected cattle that give positive result with the CFT (Rose and Roepke, 1957). The low PH (+3.6) of the antigen enhances the specificity of the test. The temperature of

the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity of the RBPT (MacMillan, 1990).

1.2.8.2.5.5 Anti-globulin (Coombs) Test

The anti-globulin (coombs) test detects (incomplete Brucella) antibodies of the Ig G_2 type and used to confirm SAT results (Hill, 1963). The coombs test, although laborious, is particularly important when the SAT is positive and CFT results are negative or inconclusive (Kiss, 1971). However, combs test results are indicative for infection only when its titres are at least two times than titres of the SAT (Hill, 1963). This is the test main limitation, as not all infected cattle show this ratio. The 2-mercaptoethanol and the rivanol tests detect specific IgG (Rossi and Cantini, 1969) and are usually used to differentiate between infected and vaccinated cattle.

1.2.8.2.5.6 Enzyme-linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is a highly sensitive method used for serological diagnosis (Sutherland, 1985). The ELISA has proven to be specific and as sensitive as the MRT and SAT in detecting Brucella antibodies in milk and serum. (Neilsen *et al* 1981). ELISA results are usually in agreement with CFT results (Ruppanner, *et al.*, 1980; Bercovich and Taaijke, 1990). The test can be used for screening and confirmation of brucellosis in both milk and serum. However, depending on the presence of traces of colostrums in the milk, or the presence of low concentration of lacted immunoglobulin ELISA may test false positive or false negative (Bercovich and Taaijke, 1990; Kerkhofs *et al*; 1990). It seems that the ELISA is less sensitive than the CFT, as some infected cattle that test positive with

the CFT may test negative with the ELISA (Cargill *et al.,* 1985; Sutherland 1984). Some researchers imply that the main advantage of the ELISA when compared with the CFT lies in its relative simple test procedure (Sutherland; *et al;* 1986). The assay is very costly when a few samples are tested, therefore, it is unsuitable for testing individual animals but it is the ideal test for screening purposes.

1.2.8.2.6 Molecular Methods

1.2.8.2.6.1 Polymerase Chain Reaction

Recently the polymerase chian reaction (PCR) has been shown to be available method for detecting DNA from different fastidious and non cultivated agent. Although there are several studies on Brucella DNA detection by PCR from pure culture, there are a few studies in the cattle have been performed with clinical or field samples. In addition, not enough data are a available to asses the performance of the PCR assay on milk samples from farm animals other than cattle (Fekete *et al* ., 1992; Romero *et al*, 1995). Recently Amel, (2005) examined 160 bovine milk samples uses PCR. She was able to detect Brucella DNA from 20 (12.5%) milk samples

1.2.9 Economic Importance

The economic loss from brucellosis in cattle arise from the slaughter of cattle herds that are infected with Brucella. Loss due to abortion or the birth of a dead calf,

or even if the calf is alive it is week . Also it resulted in decreased milk yield, retention of the placenta, impaired fertility and sometimes arthritis or bursitis. It is difficult to estimate the financial loss caused by brucellosis. As it depends on the type of cattle farming, herd, size, and whether it is an intensive or extensive cattle farm. (Abdussalam, *et al.* 1976).

Losses due to the disease in human can not be estimated. The diseases is of long duration. Characteristic symptoms lead to loss of energy and vitality, must affect the productivity of its victims. Abdussalam *et al.* (1976), Mustafa and Fawi (1968), stated that poor sector of population that keep sheep and goats have chances of exposure over 90% .

1.2.10 Control and Eradication of Bovine Brucellosis

The disease is difficult to cure because of the capacity of the organism to grow intracellulary. Because of the tremendous effects of the disease on economy and exportation, it must be controlled and eradicated. There are three essential components of the bovine brucellosis control and prevention (Plummet, 1986).

1. Protection of herds free from the disease and areas of importation from non-free areas by restriction of animal movement.
2. Vaccination of exposed herds or animals
3. Segregation of infected animals or herds from free ones and this is done by testing and slaughter or isolation of sero-positive animals

(Niccoletti, 1980) stated that maximum control and prevention is achieved when the three ways above are combined. According to the world health organization export

committee on Brucellosis (WHO, 1986) many countries has imposed measures to control or eradicate *Br. abortus* due to economic losses, and hazards to human health associated with the disease and the main approaches for control have included:

1. Detection, usually by serological methods
2. Elimination of affected animals
3. Vaccination of remaining animals
4. Observation of general principles of hygiene (Garcia, 1991)

1.2.11 Treatment of Bovine Brucellosis

Prolonged treatment of infected domestic animals with a high dosage of antibiotics is not used due to the appearance of antibiotics in human food chain and its interference with the production of milk products. Moreover, as Brucella is facultative intracellular bacteria, relapses after treatment usually occur. Therefore, efforts are directed at prevention or eradication of brucellosis (Fensterbank, 1976).

The influence of antibiotic therapy has been studied by different workers. Fensterbank (1976) showed that the cows treated with oxytetracycline has less severe infection than non-treated cows, and some were considered cured by the therapy (Hall and Hanion, 1970). The tetracycline antibiotic is the most effective and inhibit 95% of strains in a concentration of 0.02 mg/ml, and is more bactericidal for *Brucella*. (Millward *et al* ; 1984). The effectiveness of multiple injections of a combination of a long acting tetracycline (20 mg/kg body weight, i.m) and streptomycin (25mg/kg body weight, i.v.) was studied by Milward *et al* (1984) and Nicoletti *et al*. (1985). Nicoletti *et al* (1989) evaluated efficacy of liposome encapsulated sreptomycin to

treat cows naturally infected with *Br.abortus*, and observed that the most effective treatment regimen consisted of two intramamary

Infusions of streptomycin liposomes and two doses of oxytetracycline administered intramuscularly.

1.2.12 The Disease in the Sudan

Brucellosis in cattle was reported in all parts of the country and the prevalence rate was found to be higher in cattle compared to other animal species. The first incidence of bovine brucellosis in Sudan was reported from a dairy herd in Khartoum where *Br.abortus* was isolated from an aborted cow (Bennet, 1943). in 1956 brucellosis was diagnosed at Joba dairy farm after a storm of abortions. Serological tests revealed about 55% positive reactors in the herd (Dafalla and Khan, 1958).

In 1957 one year later brucellosis was serologically diagnosed in Western Sudan both in Elobied and Nuba mountains and there were 15% serological positive ones (Dafalla and Khan ,1958).

During the year 1958-1959 samples of sera and milk collected from Nisheshiba and Umbinein revealed 144 positive samples from 1345 bovine sera and 9 out of 104 bovine milk samples. (Dafalla, 1962). Elnasri (1960) tested sera collected from cattle in the Upper Nile Province. The percentage of reactors to agglutination test were calculated on a district basis and it was evident from the figures that the percentage in the Province was about 15% among cattle. Abdulla (1966) surveyed for brucellosis in Wadi Halfa District and obtained 3% positives in cattle, 1.7% in sheep while 1.5% in goats.

Mustafa and Nur, (1968) investigated brucellosis in Gash and Tokar Districts of Kassala Province in Eastern Sudan, results showed an incidence of 1.1 and 5.5% respectively. This was followed by anther survey on Kenana cattle of the Fung districts, Blue Nile Province, East and West of the Blue Nile River. The incidence in eastern and western banks was 8.7 and 5-7% respectively. Shigidi and Razig, (1973) isolated *Br.abortus* from knee hygroma of abull. Ibrahim and Habiballa, (1975) investigated the milk collected from twenty three herds in Western Sudan using milk ring test (MRT). They found that positive MRT reactions varied in different localities. In Western Sudan it ranged between 14.2% to 66.7%. From a total of 242 cows. 38% of samples were MRT positive, 4.5% were suspicious and 57.5% were negative.

Habiballa *et al.* (1977) tested 2720 cows in Khartoum, Elgezira and Nile Province and obtained in Khartoum Province dairy herds A.B.C percentages of positive reactors 0.5, 11.1 and 8.2 respectively. In Elgezira Province the percentage were 30.9, 3.1, 7.1 and 4.4 respectively in four dairy herds. And in the Blue Nile Province the percentage of positive was 1.6 in one dairy herd and the other herd was negative.

Suliman (1987) investigated prevalence of brucellosis in Khatroum and Elgezira Province in a total number of 2085 milk and 710 blood samples using SAT, RBPT and MRT, he obtained a prevalence rate (15.2%) of bovine brucellosis in the two regions according to MRT. The prevalence in the milking cows was (14.1%) by SAT. He found that there was no association between infection in dams and daughters and all bulls tested react negatively to all blood tests performed.

Musa *et al*.(1990a) studied clinical manifestation of brucellosis in the cattle of the Southern Darfur. The authors recommended elimination of cattle with hygroma from herds. In another study Musa *et al* .(1990b) under took the subject of identification of biovars of Brucella species isolated from infected cattle in nomadic, semi-nomadic and sedentary husbandry in Southern Darfur. A total of 1040 heads of cattle were examined and 20% were positive, accordingly they concluded that brucellosis was wide-spread in the area.

Musa, (1995) reported that the disease in Darfur States and Western Sudan, appears to be widely spread. The prevalence of the disease was 13,9% in cattle and 7.76% in camels.

Siddiga, (1995) investigated prevalence of bovine brucellosis in Khartoum States in a total number of 740 blood samples and 423 milk samples using rose Bengal plate test, buffered acidified plate antigen test, milk ring test and standard tube agglutination test. She obtained different prevalences rates as follow : rose Bengal plate test (1.62%) and in the milk ring test (5.48%) and buffered acidified plate antigen test (1.89%).

Raga, (2000), investigated brucellosis in camels and cattle in Darfur States. Using milk ring test, rose Bengal plate test, and serum agglutination test and complement fixation test. A total of 904 heads of camels were examined . The prevalence rate was found to be (6.2%). Hygroma aspirates from knee joints of 10 bulls in Southern Darfur were tested. All samples were found to be positive for brucellosis.

1.2.13 Zoonotic Importance

Human are infected through handling infected cows or their tissues, or through drinking infected milk. Pasteurization will prevent the disease. Milk from other animal species such as a sheep, goats and camels is an important source of human brucellosis. Severe infections occur with *Br.melitensis* (Malta fever).

1.2.14 Human Brucellosis

1.2.14.1 Definition

Brucellosis in human is called undulant fever because the fever is typically undulant, rising and falling like a wave. It is also called brucellosis after its bacterial cause. It is also alternative names Cyprusfever, Gibraltar fever, Malta fever, Rock fever. (Bardenwerper, 1952).

1.2.14.2 Reservoirs of Infection

Brucellosis is a disease of man and animals, specially man's livestock, and thus sheep, goats, cattle; pigs are important sources of human infection. (Spink, 1946). The epidemiological significance of these animal species is determined by the species of Brucella normally found in them. *Br. melitensis* (sheep and goat), is highly pothogenic for man. *Br.abortus* (cattle) is relatively less pathogenic for man. Foci of acute brucellosis in sheep or goats are often the site of epidemic outbreaks in man. In foci of bovine brucellosis sporadic cases of clinically apparent disease occur. (Feiz, *et al*. 1978).

1.2.14.2 Transmission of Brucellosis to Man

The disease is transmitted through contaminated and untreated milk and milk product and by direct contact with infected animals (cattle, sheep, goats, pigs, camels, wild ruminants and seals) and animal carcasses. (Bucknan *et al*, 1974). Transmission can be through abarsions of the skin from handling infected animals, also infection occurs more frequently by ingesting contaminated milk and dairy products. Groups at elevated risk include abattoir (slaughter house) workers, meat inspectors, animal handlers, veterinarians, and loboratory workers (Ergonul *et al,* 2004).

1.3 The Genus Brucella

Previously six species, *Br. melitensis, Br.abortus, Br.suis, Br. Ovis, Br.canis* and *Br.neotomae* were identified in the genus Brucella (Nicoletti, 1984).

1.3.1 Taxonomy

The species identification of the genus Brucella is based on two sets of properties: lysis by phages and oxidative metabolic profiles on selected amino acid and carbohydrates substrates. This classification based on recommendations made by the subcommittee on taxonomy of Brucella of the International Committee on Bacteriological Nomenclature in 1963 and subsequently mentioned in later reports (1975, 1982 and 1984). The classical species (*Br.melitensis, Br. abortus* and *Br.suis*) may be classified to the biovar level by four main test: Co_2 requirement, H_2s production,dye (thionin and basic fuchsin) sensitivity and agglutination with mono

specific A and M antisera (Alton *et al.,* 1988). Corbel (1990) mentioned that, there was close relationship between the oxidative metabolism and phage lysis patterns and that both procedures were useful for identification of the species.

According to Bergey's Manual (1984), the members of the genus are currently classified into six species with their biovars. *Br.melitensis* was further divided into three biovars. *Br. Abortus* into nine, *Br.suis* into five, but *Br.neotomae*, *Br.ovis* and *Br.cains* have no biovars identified (Alton *et al* ., 1988; Corbel, 1990). *Br.melitensis, Br.abortus*, *Br.suis* and *Br.neotomae* occur in smooth phases especially on primary isolation. While *Br.ovis* and *Br.canis* occur in rough forms.

1.3.2 Morphology

Members of the genus Brucella are Gram-negative, non-motile and do not from spores and a capsule. They are coccobacilla or short rods, the cell varies from 0.4 to 1.5 um in length and 0.4 0.8 um in width. The organisms arranged singly and less frequently in pairs, short chains or small groups (Spink, 1986).

1.3.3 Cultural and Biochemical Characteristics

The organisms are aerobic but many strain reguire supplementary Co_2 for growth especially on primary isolation. Growth is slow and is usually visible after 48 hours of incubation at 37°C. Colonies usually 0.5-1.0 mm in diameter, transparent, raised, convex, with an entire edges and soomth glistening surface. *Br.canis* and *Br.ovis* characteristically produce non smooth colonies, non smooth variants of the other species also occur.

According to Bergey's Manual of Systemic Bacteriology (1984), most strains require complex media containing several amino acids, thiamin, nictoninamideions, some strains may be induced to grow on minimal media containing an ammonium salt as the sole nitrogen source. Growth is improved by serum or blood. Enriched media such asd serum agar, liver infusion dextrose, potata and glycerol potata are recommended for primary isolation and optimum growth (Buxton and Fraser, 1977). Some strain of Brucella require the presence of serum in the medium for their growth especially on primary isolation, serum dextrose agar, serum , tryptose agar and serum , tryptocase soy agar are recommended as the best basal non selective media (Alton, *et al.*, 1988).

1.3.4 Resistance to Physical and Chemical Agent

The members of genus Brucella are sensitive to heat and are killed by pasteurization or by exposure to 60°C for 30 minutes. It is readily killed by Ultra violet or Gamma ray's under complete exposure the organisms are susceptible to an acid PH, disinfectant and direct sunlight (Buxton and Fraser, 1977).

1.3.5 Survival of the Organisms

The survival of the organism in environment may play a role in the epidemiology of the disease. Wary (1975), reviewed many studies conducted to determine the ability of Brucella organisms to survive under various experimental and environmental conditions. Temperature, humidity, and PH influence the organism's ability to survive in the environment. Brucella are sensitive to direct sunlight, disinfectant and pasteurization. In dry conditions, they survive only if embedded in protein (Davies

and Casey, 1973). Brucella can survive in tap water for several months at 4-8°C, 2.5 years at 0 °C, and several years in frozen tissues or medium. Brucella can also survive up to 60 days in damp soil, and up to 144 days at 20 °C and 40% relative humidity. Brucella can survive 30 days in urine, 75 days in a borted fetuses and more than 200 days in uterine exudates. In bedding contaminated with infected faecal material Brucella will be destroyed at 56 °C-61 °C within 4.5 hours (King, 1957). However, there are conflicting reports as to its survival in liquid manure. A according to one study *Br.abortus* can survive at least 8 months at 12 °C. Elberg, (1981) concluded that Brucella can be recovered for a long time from refrigerated meat.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Samples

2.1.1 Types and Sources

A total of 120 sera and 113 milk samples were collected from bovine dairy farms in Kosti area. Another 100 sera were collected from patients with different clinical manifestations presented to Kosti hospital.

2.1.2 Collection

2.1.2.1 Serum Samples

Blood samples from cattle for sera preparation were taken as described by (Alton *et al*.,1975). The skin over the jugular vein was rubbed with 70% alcohol and disinfected by the application of tincture of iodine. Then 10 ml blood was withdrawn using a labeled vaccutainer. Samples were put in a wire basket under shade, before taken to laboratory with minimum possible shaking. These samples were kept overnight at 4 C° in a refrigerator to separate the serum. Some time the blood sample centrifuged at 2500 rpm for five minute to separate the serum.

A ready prepared serum samples were collected from random patient, presented to Kosti Hospital with different clinical manifestations.

2.1.2.2 Milk Samples

Milk was collected from milking cows according to Alton *et al* (1975). The whole udder was washed and dried and the tip of each teat was disinfected with alcohol and wiped dry. The first one or two streams of milk were discarded, and then 20 ml of composite sample was taken from each cow directly into a labelled sterile universal bottle. The samples were put into an ice box and then transported to the laboratory. Milk samples were kept in the refrigerator until used within 24 hours.

2.2 Rose Bengal Plate Test (RBPT)

All bovine and human sera were tested for presence of brucellosis using (RBPT). The test was done as described by Morgan *et al*., (1978) as follow:

1. Capillary tube action was allowed to draw serum up in to the backline (0.03 ml).
2. The antigen was placed on tear-drop space by covering the hole and then compressing the bulb.
3. Antigen (0.03 ml) was placed adjacent to the serum. The antigen was well mixed and the dispenser held vertically.
4. The serum was mixed with antigen with the broad end of a clean stirrer and spread over the entir surface of the tear drop test area.
5. The card was rocked slowly (about 12 times per minute) for four minutes.
6. Result was read as follows:
    a. Negative when there was no agglutination or clumping, or showing a pattern of dispersed particles without clumps.

b. Positive when there was agglutination, with moderate to large clumps

2.3. Milk Ring Test (MRT)

All milk samples were tested for brucellosis using milk ring test (MRT). At the same time they were tested to demonstrate Brucella organism using modified Ziehl Neelsen stain (MZN).

The test was done according to, Morgan *et al* ., (1978).as follow:

Milk samples and the antigen are removed from the refrigerator and left at room temperature of an hour before conducting the test.

1. The milk sample was shaken gently to ensure even disruption of cream.
2. One ml of milk was pipetted into an agglutination tube.
3. One drop of antigen (0.03 ml) was added by standard dropper.
4. Contents were mixed gently, and then incubated at 37°C for one hour.

5.Results were recorded as follows:

Colour of cream ring	**Colour of milk column**	**Degree of reaction**
Definitely stained blue	White	++++
Definitely stained blue	Slightly blue	+++

Slightly more blue	Definitely blue	+
White	Definitely blue	-

2.4 Modified Ziehl Neelsens Stain (MZN)

This is special stain used for staining the bacteria of the genus Brucella.

2.4.1 Preparation of Smears

Smears were done directly from samples on a clean dry glass slide. The smears were allowed to dry in air then fixed by gentle flaming.

2.4.2 Stain Method

The staining produce was done according to Barrow and Feiltham (1993) as follows:

1. The smear was dried and fixed over a flame
2. Then stained for ten minutes with a 1:10 carbol fuchsin (1 gm basic fuchsin dissolved in ten ml absolute ethanol solution).
3. Then washed with tap water
4. Then differentiated with 0.5% acetic acid for not more than 30 seconds
5. Then washed thoroughly with tap water
6. Then differentiated lightly with 1% methylene blue (20 seconds). The organism stained red with blue background.

2.5 Sterilization

Glasswares such as test tubes, pipettes, flasks were sterilized in the hot air oven at 160 C° for 90 minutes. Bottles were sterilized in the autoclave at 15 Ib./in ^{2} for 15 minutes (121°C) .

2.6 Data Collection

Data on herd number, herd size, milking hygiene, feeding and drinking hygiene, history of abortion, and vaccination program were collected from the examined dairy farms in Kosti area.

2.7 Statistical Analysis

Microsoft excel (Window 2003) and stata 6.0 for windows 98/95/NT were used for data analysis. Chi-square (X^2) was used to assess relationship between some factors and presence of bovine brucellosis. To quantify the statistical significance, the Odds Ratio (OR) was employed. The factor could be a risk for the disease when the OR greater than one.

Sensitivity and specificity were calculated to evaluate rose Bengal plate test (RBPT) and milk ring test (MRT) based on modified Ziehl Neelsen stain (MZN) (Gold standard for detection of bovine brucellosis).

CHAPTER THREE

RESULTS

Out of 113 milk samples examined based on modified Ziehl Neelsens stain, 14 (12.39%)were demonstrated the organism. The seen organisms showed acid fast bacilli, pink cell with blue background. While milk ring test (MRT) revealed 18 positive (15.93%) out of 113 milk samples. Positive samples showed formation of clear blue ring at the top column of the milk in the test tube.

Out of 120 bovine serum samples 12(10%) were positive using rose Bengal plate test. The positive samples showed varied degrees of agglutination varied from + to +++++.

From the 18 positive milk samples by MRT 14 were demonstrated the organism with (MZN). 7 milk which were positive by MRT and MZN, their crosponds serum samples were positive by RBPT. While the milk samples which were positive by MRT their crosponds serum samples were positive by RBPT . Table (1) shows results of different tests.

The Prevalence of Brucellosis in Human

The prevalence of human brucellosis in Kosti area based on rose Bengal plate test (RBPT) was 2% (out of 100). (Table 2)

Table 1: Prevalence of brucellosis based on different tests from dairy farms in Kosti area

Test	Total No. examined	Positive (%)	Negative (%)

MZN	113	14 (12.39%)	99 (87.61%)
RBPT	120	12 (10%)	108 (90%)
MRT	113	18 (15.93%)	95 (84.07%)

Table 2: prevalence of human brucellosis from Kosti Hospital

Test	**Total**	**Positive (%)**	**Negative (%)**
RBPT	100	2 (2%)	98 (98%)

Risk Factor Analysis For Presence of Bovine Brucellosis

Positive correlation was found between history of abortion and occurrence of bovine brucellosis in dairy farms in Kosti area (X^2= 33.55, P<0.01). According to the results of Odds Ratio (OR= 32), this history of abortion could be a risk factor for presence of bovine brucellosis. (Figure1).

There was no relationship between number of calves and occurrence of bovine brucellosis (X^2= 3.98, P> 0.05). Also the status of the hygiene was statistically not significant with presence of bovine brucellosis (X^2= 0.69, P> 0.05). The results are summarized in Tables (3 and 4).

Table 3 : Some factors related to bovine brucellosis

Unit	Frequency %
Sex Male female	 7(5.83%) 113(94.17%)
Number of calves 1-3 4-6 >6	 50(44.25%) 40 (35.40%) 23 (20.35%)
History of abortion Yes No	 10 (8.85%) 103 (91.15%)
Hygien of farm Good poor	 2 (40%) 3 (60%)

Table 4: **The relationship between number of calves and hygiene of farms with presence of bovine brucellosis in dairy farms in Kosti area.**

Factor	Chi-square (X^2)	P. value
Number of calves	3.98	0.136
Hygiene of farms	0.69	0.40

p. value for both factors was statistically not significant (P> 0.05).

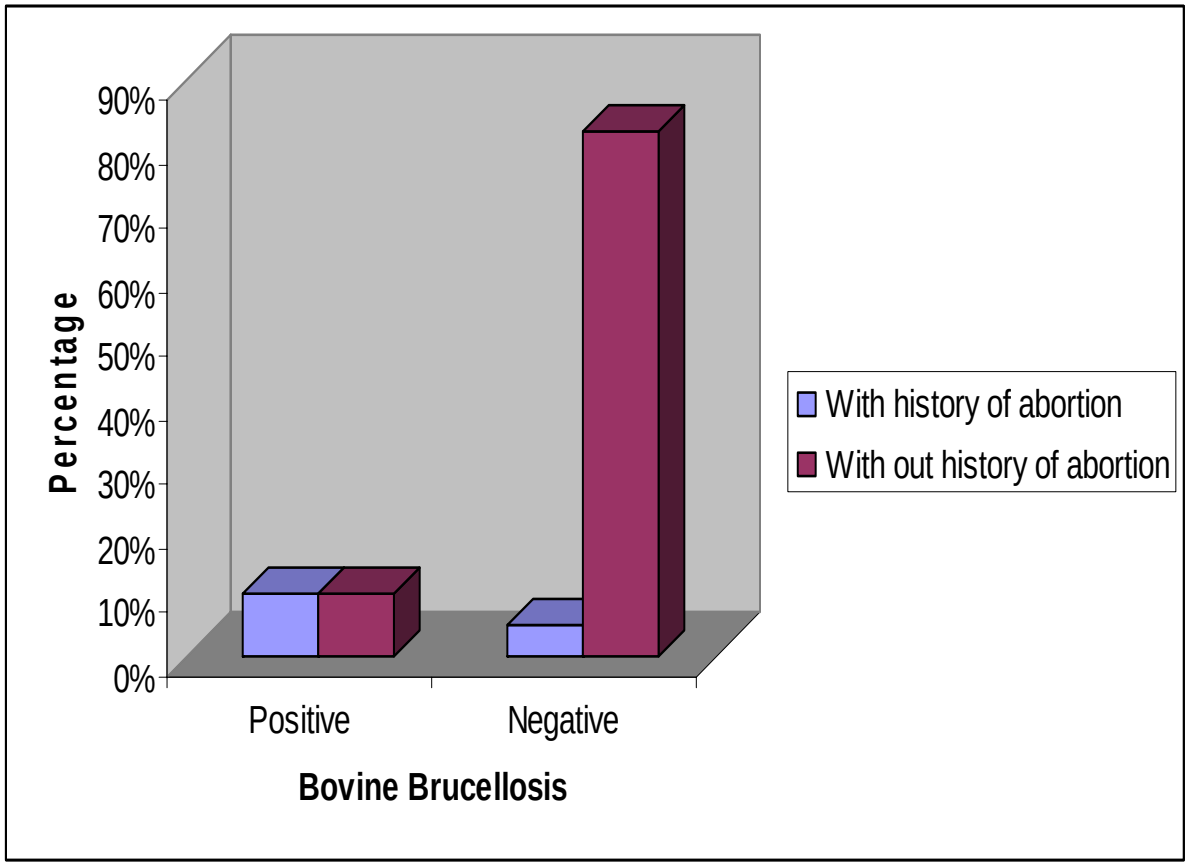

Figure 1: The relationship between abortion and occurrence of bovine brucellosis

Sensitivity and Specificity of (RBPT) and (MRT) for Detection of Bovine brucellosis.

Based on modified Ziehl Neelsen stain results high sensitivity (100%) and high specificity (95.96%) were recorded for milk ring test (MRT) for detection of bovine brucellosis.

In contrast, low sensitivity (50%) was reported for rose Bengal plate test (RBPT). However, the specificity was relatively high (95.96%) for the same test for detection of bovine brucellosis. The results are shown in Table 5.

Table 5: The sensitivity and specificity of rose bengal plate test (RBPT) and milk ring test (MRT) based on modified Ziehl Neelsen stain (MZN).

Test	**Sensitivity**	**Specificity**
Rose Bengal Plate Test (RBPT)	50%	95.96%
Milk Ring Test (MRT)	100%	95.96%

Agreements Between Different Tests Used for Detection of Bovine Brucellosis.

Prefect and high agreement (Kappa statistic= 0.85) was observed between modified Ziehl Neelsen stain (MZN)and milk ring test (MRT). While a modrate agreement (0.56) was recorded for rose Bengal plate test (RBPT) and milk ring test (MRT). The same moderate agreement (0.50) was obtained for modified Ziehl Neelsen stain (MZN) and rose Bengal plate test (RBPT). The results are summarized in Table 6.

Table 6: Agreements (Kappa statistic) between different tests used for detection of bovine brucellosis

Test	**Agreement (%)**	**Kappa statistic**
MZN and RBT	80.29%	0.50 a
MZN and MRT	75.63%	0.85 b
RBPT and MRT	77.44%	0.56 a

a= indicated moderate agreement b= indicated prefect agreement

CHAPTER FOUR

DISCCUSSION

This study was planned to investigate brucellosis in both man and bovine dairy farms in Kosti area. The results revealed that the prevalence of human brucellosis was (2%) based on rose Bengal plate test (RBPT). While, the bovine brucellosis given a prevalence of (12.39%) based on modified Ziehl Neelsen stain (MZN).

In this study, the prevalence of bovine brucellosis was relatively high (12.39%) in dairy farms examined area. This may be attributed to the fact that the farms were lacking the culling practice. Moreover, the status of the hygiene in the farms was not so good and there was no strategy for vaccination in all farms in our study.

On the other hand, our study revealed that brucellosis was also present in human (2%) in Kosti Hospital. Patients records showed that they were in contact with cattle according to their jobs in the dairy farms. This finding agreed with Ibrahim (1990), who explained that man can be infected with brucellosis through direct contact with animals or by ingestion of animal products which contaminated with *Br.abortus.* Further more, he confirmed that raw infected milk, fresh cheese and other milk products made from infected raw milk carry the risk of brucellosis. In contrast, samples from workers in the examined farms were negative.

As seen from the results, high sensitivity and specificity were obtained for milk ring test (MRT) (100% and 95.96%, respectively). While, rose Bengal plate test

(RBPT) gave high specificity (95.96%) and low sensitivity (50%). It is well known that serological diagnosis of bovine brucellosis is considered to be unreliable when applied during the period of 2-3 weeks after abortion or calfing (Rodositis *et al;* 2000). The same author explained that low sensitivity of rose Bengal plate test (RBPT) against brucellosis in both adult and calves is most likely due to vaccination and clostrum resulting in false positive due to antibody activity from vaccination as well as clostral antibody in calves. On the other hand, false negative reaction for rose Bengal plate test (RBPT) is attributed to early incubation of the disease and immediately after abortion. However, the RBPT is an excellent test for large-scale screening of sera. Furthermore, (Radostits *et al.,* 2000) stated that the major problem in brucellosis eradication programs is the false positive reactors. our results regarding high sensitivity and specificity of MRT is agreed with Radostits *et al* (2000) who explained that the MRT is the satisfactory for the surveillance of dairy herds for bovine brucellosis.

The results revealed that there was no association between number of calves and presence of bovine brucellosis (X^2=3.98, P>0.05). This finding is agreed with Enrigh (1990) who, reported that brucellosis occurred in cattle of all ages.

A negative correlation was observed between hygiene of farms and occurance of bovine brucellosis which is surprising. This result disagreed with (Blood *et al.,* 1989) who, found strong relationship between hygiene of farms and management and presence of bovine brucellosis. A positive correlation was obtained between history of abortion with regard to the presence of bovine brucellosiss. Simallary, Corbel, (1989)

found that an out break of bovine brucellosis in dairy farm is strong related with the cases of the abortion in the farms.

All bulls in the five examined farms found to be negative for brucellosis except one farm in which the bull used for insemination was positive. The prevalence in this farms was found to be high. Although bulls play less important role in the spread of infection but can spread infection by semen used for artificial insemination (Blendixen and Blood, 1947). The high prevalence in this farm may be attributed to this bull.

CONLUSIONS

1. Brucellosis in both man and cattle is present in Kosti area.
2. High sensitivity and specificity observed for MRT for detection of bovine brucellosis. In contrast, RBPT gave low sensitivity. However, relatively high specificity was obtained for the RBPT.

3. History of abortion for a cow is most likely associated with the presence of bovine brucellosis and can be regarded as risk factor for the disease.

RECOMMENDATIONS

1. An extensive investigation is required in order to clarify epidemiology of human and bovine brucellosis as well as the various related risk factors in Kosti area.

2. Vaccination of healthy cattle as well as culling practice for positive cases are needed for control and eradication of bovine brucellosis in studied farms
3. Extension programs on brucellosis are need for farm workers.

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