Detection of Antibodies against Bovine Brucellosis in ElHawata area, ElGadarif State

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Dedication

To the soul of my mother .................
The greatest love, .................................. 
Whoever her effort made this work, but 
unfortunately she doesn’t see it. 
To my father ......................................
To my brothers, sister ............................
I dedicate this work.
Preface

This work was carried out at the Department of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Science, University of Khartoum Under Supervision of Dr. Ahmed Zaki Saad.
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Abstract

The main objective of the present research was to study Bovine brucellosis in ElHawata area, ElGadarif State. A total of 121 bovine sera were collected randomly from cattle of different age and breeds. Data regarding the history of the disease (frequent abortion) in the area were recorded. Samples were 1st subjected to serological investigation using Rose Bengal Plate Test (RBPT). 19 samples (15.7%) were found positive for antibodies against Brucellosis. The result was confirmed with c-ELISA. The result was almost identical with RBPT except one sample which was found positive with c-ELISA. Only positive samples were tested with Serum Agglutination Test (SAT). the result confirmed that of c-ELISA and revealed antibody titre ranged between 17-1280. Different age groups were included in the study and the disease was detected in all groups. The disease was more prevalent in cross breeds.

It was concluded that Bovine brucellosis is prevalent in ElHawata area, ElGadarif State.
ملخص الاتروحة

توجد 5 حالات من تسمم البروسيليا في المنطقة، حيث تم الكشف عن 2 حالة جديدة. كانت نسبة الوفيات 12.2% من الحالات المعازبة.

إليك بعض النتائج:

- نسبة الوفيات (%) 5.7
- نسبة الكشف القدرة (19%) للكشف عن البروسيليا
- نسبة الكشف الجثاثي (419%) ومتراقبة (19%)
- نسبة الكشف العيني (15.7%)
- نسبة الكشف الفوري (19%)
- نسبة الكشف المبكر (19%)

Introduction

Brucellosis is a zoonotic disease with public health and economic implications. Losses in animal production due to brucellosis include diminution of milk and meat, abortion, infertility, longer calving intervals and higher culling rates (Blood et al, 1983). The disease poses great hazard to human health specially in countries like Sudan where no proper programme for disease control and the microbiological quality of milk is rarely checked. The problem exacerbated with the intensification of production system around urban area and major towns with increased movement of highly producing cattle between states and towns. Also brucellosis was reported among local breeds reared under nomadic and semi-sedentary production system. The hazard of the disease is not limited to groups working closely with cattle such as veterinarian, animals attendants, pastoralist, butchers, but extend to the consumer to whom raw milk is delivered in a traditional way without pasteurization; a considerable proportion of the population takes raw milk without pasteurization or boiling. Furthermore milk is processed into cheese in many areas of the country without any attempt of pasteurization or boiling.

The first isolation of Brucella organism from animals was made by Bang (1897), who was the first to report contagious abortion in cattle and other animal species and he named his isolate Bacillus abortus, which was followed by other names, Corynebacterium abortus, Bacterium abortus and Alcaligenes abortus. Meyer and Shaw (1920) suggested the name Brucella for the genus.
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 (Dafaala, 1962). Thereafter the disease was detected in many parts of the country following isolation of the causative agent or antibody detection.

Detection of brucella antibodies is a useful method for diagnosis of bovine brucellosis in many countries in the final phase of an eradication program, and it is still used for monitoring when countries are certified officially free of brucellosis.

Although brucellosis was extensively studied in the country, epidemiological data regarding some parts of the country are still lacking.

The objective of the present study was to investigate ElHawata area (Elgadarif state) for bovine brucellosis. ElHawata represent an important area in the epidemiology and spread of diseases why its joints three different states and its major polls of animals in the summer. Bovine serum samples were investigated for antibodies against Brucellosis using Rose Bengal, competitive ELISA and serum agglutination test.
CHAPTER ONE
LETRATURE REVIEW

1. The Genus Brucella

1.1. Morphology

The brucella organism appears as cocci, coccobacilli or short rods measuring 0.5-0.7 µm in diameter and 0.6 - 1.5 µm in length. In stained smear the organism appears single, in pairs, chains or small groups. They are non-motile and do not form spores and capsule.

1.2. Cultural and biochemical characteristics

The organism is aerobic but some strains require CO₂ for primary isolation. Growth is slow and is usually visible after 48 hours of incubation at 37°C. Colonies are about 0.5 mm in diameter and appear round, convex with smooth glistening surface. The recommended enriched media for primary isolation and optimum growth include serum agar, liver infusion, dextrose potato, glycerol potato and Brucella agar (Buxton and Fraser, 1977). On blood agar, colonies are usually 0.5 – 1.0 mm in diameter, raised and convex, with an entire edges and smooth shiny surface. *Br. canis* and *Br. ovis* produce non smooth colonies. Non smooth variants of the other species also occur (Patrick et al, 2003).
1.3. Molecular biology

All members of the genus *Brucella* produce similar patterns on basic electrophoresis of acid phenol soluble protein (Corbel, 1990). Ribosomal-Ribonucleic acid DNA hybridization studies have indicated that the genus *Brucella* is related genetically to *Agrobacterium*, *Mycobacterium*, *Phyllobacterium*, *Rhizobium* and belongs to ribosomal Ribonucleic acid upper family IV (Deley et al, 1983).

The genome of *Br. abortus* biovars has two circular chromosomes. The first chromosome is 2, 124, 241bp long with a G+C content of 57%. The second chromosome is 1, 162, and 204 with a 57% G+ C content. This species is able to cross the species barrier and affects both livestock and humans (Delvecchio et al, 2002).

1.4. Antigenic relatedness

The genus *Brucella* is highly characterized by having o.chain poly saccharide antigens which have recently been characterized at the molecular level in *Br. abortus* by Pery et. al. (1986). The structural characteristic (N-acylated 4.amino -4, 6-dideoxy-D-mannose repeating uints in the o.chain) also exist with o.chain of some other Gram-negative bacteria which allow antibody cross reactions. The known cross reacting species or strain are *Yersinia enterocolitica* serogroup O:9; *Salmonella* serotype of kuffman –white group N:30; *E.coli* O:157 and O:116 serotype; *Pseudomonas maltophilia* and *Vibrio cholerae*. This potential for cross reaction complicate the use of anti LPS serum as a diagnostic agent unless the presence of other known cross reacting species (Nielsen and Duncan, 1990). However, DNA homology studies have shown that members of the genus *Brucella* lack
homogenicity with other microorganism having similar guanine-cytosine ratio like *E. coli, Agrobacterium tumefaction* and *Serrotia marcescence*.

1.5. Susceptibility to phages

Over 40 Brucella phages have been reported to be lytic for Brucella members: All phages are specific for the genus *Brucella*, and are not known to be active against any other bacteria that have been tested. Thus, lysis by Brucella phage is a useful tested to confirm the identity of *Brucella* species and for specification within the genus. The Brucella phages currently used for Brucella typing are: Tbilisi (Tb), Weybridge(Wb), Izatnagar (Iz) and R/c. The three former phages are used for differentiation of smooth *Brucella* species. R/c is lytic for Br. *ovis* and Br. *Canis*.

1.6. Susceptibility to dyes and antibiotics

Susceptibility to the dyes, thionin and basic fuchsin (200ug/ml) which varies between biovars is one of the routine typing tests of *Brucella*.

On primary isolation, *Brucella* is usually susceptible in vitro to gentamicin, tetracyclines and rifamycine. Most strains are also susceptible to ampicillin, chloramphenicol, erythromycin, kanamycin, novbiocin and streptomycin .But variation in susceptibility may occur between species, biovars and strains. Most strains are resistant to B-lactimins, cephalosporin, polymyxin, nalidixic acid, amphotercin B, cyclohexamide, lincomycin, nystatin and vancomycin at therapeutic concentration .(Alton *et. al.*, 1988).
1.7. Taxonomy of the genus *Brucella*

The old classification of the genus (and relevant nomenclature) into six species, *Br. melitensis*; *Br. abortus*; *Br. suis*; *Br. neotomae*; *Br. ovis* and *Br. canis* (Gorbel and Brinly-mogan, 1988), is the classical world wide. The first four species are normally observed in the smooth form, where as *Br. ovis* and *Br. canis* have only been encountered in a rough form. Seven biovars are recognized for *Br. abortus* (1-6 and 9), and 5 for *Br. suis* (1-5). However *Br. abortus* biovar 8 no longer exist (Mayer and Morgan, 1973) and *Br. abortus* biovar 7 was reported to be mixed culture of *Br. abortus* biovar 3 and 5 (International committee on systemic bacteriology sub committee on taxonomy of Brucella, 1986). As a result both Biovars were not included in recent classification (Alton *et al*; 1988 and Corbel, 1990). However DNA – DNA hybridization studies have shown that only one species *Br. Melitensis* exist in the genus and the other species were actually biovars (Verger *et. al.*, 1985). Different species (biovar) have different hosts, sheep and goat are primarily host for the (*Br.melitensis* biovar ovis) dogs for (*Br. melitensis* biovars canis) and wood rat lepidthomas for (*Br. melitensis* biovar neotomae). (Corbel *et. al.*, 1990). The different spp. could infect organ of secondary host except *Br. neotomae*, which is presently known to infect only desert wood rat (Corbel and Hendary, 1983). These species and biovars are defined by their host specification, tolerance to function and thionin, CO₂ requirement, rate of urease activity, agglutination in specific adsorbed rabbit anti serum and susceptibility to *Brucella Tbilisi* phage (Weyant *e.t al.*, 1996).
1.8. Survival of Brucella in the environment

Compared with other non sporing pathogenic bacteria, *Brucella* has a relatively high capacity to survive and persist in the environment under suitable conditions. Numerous studies have assessed the persistence of *Brucella* under various environmental conditions. Thus when pH>4, high humidity, low temperature and absence of direct sunlight, *Brucella* may retain infectivity for several months in water fetal membrane of aborted foeti, feaces and liquid manure, wool, hay, on building, equipments and clothes. *Brucella* are able withstand drying particularly in the presence of extraneous organic material and remain viable in dust and soil. Survival is prolonged at low temperature, specially bellow 0°C (Alton, 1985; Joint FAO/WHO committee, 1986; Nicoletti, 1980). The organism is susceptible to an acid pH, disinfectants and direct sunlight.

Survival of *Brucella* in milk and dairy products is related to a variety of factors including the type and age of product, humidity level temperature, change in pH, moisture content, biological action of other bacteria present and condition of storage. *Brucella* does not persist for long time in ripened fermented cheese. The optimal fermentation time to ensure safety is not known, but is estimated at 3 months. (Nicoletti, 1990). However in normally acidified soft cheese. The strictly lactic acid and short-time fermentation and drying increase the survival time of *Brucella*. Previous pasteurization of milk or cream is the only means to ensure safety of these products, the survival time of *Brucella* in meat is short, except in frozen carcases where the organism can survive for a year. The number of organisms per gram of muscle is small and rapidly decreases with pH drop of the meat.
Br. suis has been isolated from hog carcasses after 21 days of refrigeration. (Manson-Bahr and Apted, 1982).

2. Brucellosis

Brucellosis is a zoonotic contagious bacterial disease caused by the members of the genus Brucella. (Corbel and Hendary, 1983). The inclusive name brucellosis of the disease was named after Bruce (1887) who reported the first isolation of Br. melitensis from human spleen. The disease in animals is characterized by bacteremia followed by location of the organism in the reticuloendothelial tissues, reproductive organs and sometimes joints. Lesion of the reproductive tract of the pregnant female in cattle, sheep and goat may result in death and abortion of the fetus. Brucella also causes lesion in the male reproductive organs in cattle, sheep, goat and dogs and also bursitis in horses. (Gillespie and Timoney, 1981). In man, Brucella melitensis causes a severe disease characterized by undulating fever, chills, headache, pain in legs, large joint and lumber region, profuse nocturnal sweating, insomnia and some times laryngitis and bronchitis (VanDerHoeden, 1964). Brucellosis is still a major problem, widely distributed throughout the world, mainly in developing countries due to traditional feeding habits and the failure to maintain standard of hygiene, because of socio-economic conditions (Ozekicit et al., 2003). Prior to the use of the name Brucellosis, the disease in animals was known by many names; such as infectious abortion, Bang’s disease, slinking of the calf and contagious abortion.
2.1. Economic importance

Animal Brucellosis poses a barrier to trade of animals and animal products; it could seriously impair socio-economic development, especially for livestock owners. (Corbel, 1973).

Brucellosis causes physical and psychological suffering, farmers suffer loss of income due to abortion, the consequent decrease in milk yield, culling of infected animals, rejection of exported consignments containing infected animals and prolonged fattening time. The country incurs costs generated by prophylactic activities, control and eradication program, hospitalization of human patient, cost of research, loss of work or income and failure in financial investment (Chaukwa, 1987).

2.2. Epidemiology

Epidemiology is the study of host-parasite relationship in population (Nicoletti, 1984). Factors which contribute to risk of exposure depend largely on husbandry practice, inter herd transmission, movement of animals, vaccination level, herd size, population density and the method of housing, contamination from wildlife source should also be considered (Grain-Bastu, 2003).

*Brucella* species infections are characterized by a marked preferred animal host specification. Different species and biovars have different hosts (Verger, *et. al.*, 1985 and Corbel, 1990). Different species could also infect a range of secondary hosts except *Br. neotomae* which is presently known to infect only one host (Corbel and Hendary, 1983).
The primary hosts act as reservoirs of infection for each particular species, while the secondary ones usually play little part in the maintenance or spread of the disease. (Corbel and Hendary, 1983). However, in cattle beside *Br. abortus* infection; *Br. suis* and *Br. melitensis* have been reported (Garcia, 1990).

### 2.3. Geographical distribution

Brucellosis occurs world wide in domestic animals. While the disease has been eradicated in most industrial countries, especially in Europe, through intensive schemes of control and eradication, its occurrence is increasing in the developing countries in an even aggravating epizootological situation. This depends on the policy of many developing countries of importing exotic high producing breed without having the required veterinary infra structure and the appropriate level of development of the socio-economic situation of the animal holder. Furthermore, the increasing international animal trade with increasing movement of animals and the trend towards intensification of animal production favors the spread and transmission of disease. Even highly developed countries like USA and France have so far not been able to eradicate brucellosis completely. In intensive dairy production systems of the tropics, an incidence of infection of up to 80% can be found. In the extensive production system of the Sahel an average disease incidence of 20 – 30% has been reported (Seifert, 1990).
2.4. Brucellosis in Sudan

In 1953, 885 sera were examined for brucellosis. Positive reactors counted for 50% from sheep, 38% from goats and 26% from cattle.

*Br. melitensis* was isolated from the milk (Dafalla and khan, 1958). In 1966 brucellosis was diagnosed at Juba diary farm after storm of abortion (31 calves out of 149 pregnancies). Serological reactions revealed that about 55% of the animal in the herd (of 358) were positive (Dafalla and Khan 1958). In 1959 brucellosis was serologically diagnosed in western Sudan both in Elobeid diary herd and among nomadic arab cattle in Nuba mountains and out of 221 samples tested, (15%) were defined positive (Dafalla and Khan, 1958).

Elnaseri in (1960) diagnosed the brucellosis serologically from 5689 serum samples collected from cattle in the Upper Nile area, southern Sudan. An investigation of bovine brucellosis in Gash and Toker district of Kassala Province in Eastern Sudan showed an incidence of 1.1 and 5.5 respectively (Mustafa and Nur, 1968). Mustafa and Hassan (1969) carried out a survey on kenana cattle of the Funj district, east and west of the blue Nile. The incidence in the eastern and western bank areas were 8.7 and 5.7 respectively. Gammel *et al*, (1987) diagnosed bovine brucellosis in nine dairy herds, out of twenty tested in Khartoum.

2.5. Transmission of disease

2.5.1. Mode of infection

According to Buxton and Fraser (1977), the disease is transmitted from infected animal or contaminated materials to susceptible one
through mucous membrane of the alimentary and respiratory tract, conjunctiva, abraded and intact skin, artificial insemination and through the vagina in some species. The dog has been shown to be mechanical and biological factors of brucellosis (join FOA/WHO report on brucellosis 1986.). Insects could also act as vehicle of infection (Corbel, 1989). In ovine brucellosis, spread of infection is mainly venereal or direct from ram to ram brucellosis can establish in the uterus of pregnant ewe, however is only carried for the term of that pregnancy (Nancy 2002). In man infection by in halation, ingestion, through conjunctiva and skin.

2.5.2. Routes of excretion and contagious material

In most circumstances primary source of dissemination of brucella is placenta, fetal fluid and vaginal discharges expelled by infected animal after abortion or full term parturition. Very large numbers of organism are shed at the time of parturition or abortion, and the excretion of the organisms from the vagina may extend from 3 weeks to several months. Shedding of the brucella is also common in udder secretion and semen and brucella may be isolated from various tissues such as lymph node from the head and those associated with reproduction and some times arthritic lesion (Alton et. al., 1988).

2.6. Susceptibility to disease and host factor
2.6.1. Age

Susceptibility to brucellosis increase with sexual development and pregnancy. Cunning ham (1977) found weak and transient titer among young heifers exposed to virulent strain of B. abortus. Suliman (1987) showed that calves were least susceptible to infection while prevalence
in lactating cows was the highest among different age groups. Calves may acquire infection in uterus or by ingestion or contaminated vaginal discharge or milk. This infection was thought to be temporal, but recent reports showed that heifer calves which were infected at early live were negative to serological test and aborted or had infected calving during the first pregnancy. These were referred to as latent carriers (Cunningham, 1997a). Suliman (1987) showed no association between infected dams and the infection within their daughters. The previous author found the possibility for infection among daughters, which are born by infected dams was 92%. Plummet et.al., (1988) found the infection of the daughter born to infected dam was 21%. Nagy and Hignett (1967) showed that the neonatal infection led to a degree of immunity against subsequent exposure to infection.

2.6.2. Sexes

Bulls are more resistant to brucella infection than sexually mature heifers and cows (Nicoletti, 1980). All bulls tested in farms where brucellosis was prevalent gave a negative serological test (suliman1987). Thomsen (1950) showed that bulls play a less important role in the spread of the disease. During the acute phase of infection, infected bulls can excrete brucella organism, but this excretion may cease when the infection become chronic (Manthi, 1965).

Bendixin and Blood (1947) suggested that the disease could be widely spread by infected semen used for artificial insemination. Manthei (1951) showed that intrauterine insemination produce a high level of infection if compared with mid-cervical insemination of semen containing virulent B. abortus strain, this may be due to the presence
of cationic proteins on the cervical mucus which protect the uterus from the invading organism.

The incubation period in brucellosis is affected by several factors such as, gestation, exposure, dose, age, vaccination and other unknown host–resistance influence (Nicoletti, 1980). Thosman (1950)) showed that the length of incubation period was inversely proportional to the stage of fetal development and time of exposure. He observed an incubation period, which varied from 53 to 252 days. The variable incubation period and the difficulties in early diagnosis of infection are among the most serious technical problems in the epidemiology of brucellosis.

2.6.3. Resistance of the host and persistence infection

The brucella spp. are intracellular parasites, so they have protection from innate host defenses and their therapeutic agents. Natural or artificial infection usually persist indefinitely although about 10 -15% recover spontaneously (Nicoltti 1980).

The effect of heredity on the resistance is not completely known. Resistance to brucellosis could inherit through polygene. Fensterlauk (1976) found that, cows treated with oxtetracycline have less severed infection than non – treated cow and some where considered cured by the therapy.

2.7. Pathogenesis

The susceptibility of the animal depends significantly on their natural resistance, their age and their level of immunity and on the environmental stress. If the infection is introduced into a non infected herd in which all animal are immunologically naive to brucellosis, so
called abortion storm may occur and almost all pregnant cows will abort.

After infection of the regional lymph nodes, bacteraemia which lasts for 1-3 weeks occurs and distribute the organism to the lymphatic system, the large parenchyma and other organs and tissues. In pregnant animal, the uterus is a preferred sit of infection where it lead to necrotizing placentitis. In non pregnant animal the first infection often occur in the udder followed by the infection of the uterus later after the onset of pregnancy.

In cattle, the uterus is the center site of multiplication of the pathogen, the enhanced virulence of the brucella inside the reproductive system is supposed to be consequence of the increased level of the sugar erythrol, which is maintained in the reproductive system. A characteristic exudative and proliferative process develops in the gravid uterus starting from the epithelium of the villous of the chorion.

2.8. Clinical signs

They can be summarized in the following:
- Abortion after the seven month of pregnancy
- Birth of week calves –atypical feature of infection of game.
- Inflammation of the seminal vesicle and vesicular glands in bulls
- Epididymitis in rams
- Chronic inflammation of the epididymis in male, joints, tendon sheath, synovial bursa, especially at the corpus in cow, the
regional lymph nodes being enlarged and containing loads of brucella.

Wherever the disease is enzootic in relatively resistant animals, as it is mostly the case in the tropical savannah, abortion is rare but the infection causes typical signs, which lead to a significant reduction in productivity. Theses are late first calving, long–inter calving time, herd fertility below 60%, hygroma and comparatively low milk production.

After recovery from apparent or in apparent abortion, females are protected against a renewed infection because of the development of massive immunity, they may even become fertile again and if not, it is because of the permanent lesion in the reproductive system which may appear due to metritis. The male animal may suffer from orchitis and show symptoms of oligo and spermia.

2.9. Pathology

A yellowish, slimy layer covers the aborted fetus which may be macerated. The after birth is edematous, slushy, the effected cotyledons, or part of them are covered by sticky, ouder less, brownish exudates, and are yellowish –grey, as a result of necrosis large amount of pathogen are excreted with the evil – smelling, dirty – grey lochiae.

Microscopically numerous mononuclear cells and some neutrophils infiltrate the storms of the chorion. Though fetuses may show no gross changes, petechiae are often to be found in the abomasums and on the mucosa of the bladder of the fetus. Spleen, liver and lymph nodes are enlarged. Pneumonia with grayish white foci may be present, necrotic foci or micro granulomas in the liver, the
lymph nodes, spleen and kidney can be found microscopically. Gross lesions are not evident on the udder, though the supramammary lymph node may be enlarged, histologically interstitial mastitis is evident.

An infected bull at first show an acute febrile general reaction with heavily swollen and painful scrotum, the animal refused food and is depressed. Acute orchitis is characterized by multifocal or diffuse necrosis of the testicular parenchyma and focal necrotizing epididymitis. Microscopically the seminal epithelial cells are necrotic and descumative, and large number of the organisms are present. In the chronic stage spermatic granulomas develop in the testicular parenchyma and epididymitis in response to dead sperm. Hygroma, in particular on the carpal joins are characteristic feature of a chronic infection, but sometimes this hygroma are found on the tarsus as well. Furthermore, the regional lymph nodes and vessels are enlarged (Scientific committee on animal health and animal welfare, 2001).

2.10. Diagnosis

The most reliable diagnosis is based on isolation of Brucella from clinical specimens (Alton et al; 1988). However this procedure is not always successful, cumbersome and represent a great risk of infection for laboratory technician and cultures are not always positive when other tests are positive (Lopez-merino, 1991).

2.10.1. Isolation of the organism

Isolation of the organism by culture is attempted from the organs and lymph nodes of the fetus, the placenta, milk, vaginal discharge or uterus exudates. Isolation of the organism from udder secretion of a cow is conclusive evidence of infection (Radostitis et.al., 2000).
Growth is obtained on most commonly used media including serum-
dextrose agar, serum-tryptose agar, glycerol dextrose agar, brucella
agar and potato agar. Growth on MacConky agar is variable and has
been observed in approximately 50% of strains (Weyant et al., 1996).

2.10.2. Guinea pig inoculation

This method is successful than direct culture specially from
contaminated material. Injection is made intramuscularly inside the
thigh and the guinea pig is killed 4-5 weeks after inoculation.
Recovery of the organism from the spleen of the guinea pigs or
positive SAT at 1/10 or over is taken as evidence of infection (Musa,
1995). Typical lesions include necrotic foci in liver, spleen, lymph
node and orchitis in male guinea pig.

2.10.3. Serological tests

Serological testing is a valuable adjunct to culture based and
molecular methods for the laboratory diagnoses of brucellosis.
Demonstration of a specific antibody response can provide useful
diagnostic information and the characterization of the response
(predominantly IgM versus IgG) assist in differentiation of a cute
infection from recurrent disease (Smits et al.; 1999). It’s believed that
no single method is completely satisfactory because none of the tests
are both sensitive and specific, and has the ability to discriminate
between vaccinated from non vaccinated animals and detecting
infected animals in the incubation period (Buxton and Farser, 1977).

Briefly primary binding assays directly measure the interaction of
antibody and antigen while conventional serological tests, such as
acidified agglutination tests or the complement fixation test (CFT)
measure secondary phenomena such as the agglutination or activation of complement (Nielsen et al, 1996).

Depending on the sensitivity (i.e. the ability of a test to correctly identify animal with disease) and specificity (i.e. the ability of test to correctly identify healthy animal or animal not having disease), test can be used to screen for, or confirm disease. Traditionally, screening tests are in expensive, fast and highly sensitive, but not highly specific. Confirmatory tests are required to be both sensitive and specific (Stemshom et al; 1985). The buffered antigen plate agglutination test (BPAT), Enzyme linked immunosorbent assays (ELISA) and the fluorescence polarization assay (FPA) are appropriate screening test (OIE, WHO, 2000) since they are all highly sensitive and specific, making them ideal tests for use in international trade.

The sum of sensitivity and specificity values for each test was averaged to give a performance index (PI) and allow for a comparisons between the different methodologies.

2.10.3.1. The serum Agglutination test (SAT)

The test is widely used for the diagnosis of human and animal brucellosis. The antigen used in the test is the whole cell and the antibodies detected are those directed against the surface molecules. The SAT has been used with success for many years in surveillance and control programs for bovine brucellosis, its specificity is significantly improved with the addition of EDTA to the antigen (McMillan et al, 1985). While the CFT and RBT mostly identify immunoglobulin G1 isotypic antibody, which is predominant in the later phase of brucellosis or in chronic brucellosis, SAT response is detected in the early phase of the disease when immunoglobulin M
antibodies are elicited (Kolar, 1984). Although the SAT is less sensitive and less specific than the CFT and RBT (Alton et al; 1975), it is still being used in several countries as surveillance method.

2.10.3.2. Rose Bengal test (RBT)

This is a simple, rapid test which detects early infection and can be used as an initial screening test. Using the test is estimated to vary from 1 to 31, depending on the level of infection and vaccination history of the herd. False positive reactions are due to residual antibodies activity from vaccination, colostral antibodies in calves, cross reaction with certain bacteria and laboratory error. However the Rose Bengal test is an excellent test for the large – scale screening of sera. In heavily infected herds the test is most useful. In herd where the prevalence of infection is low and where vaccination has been used this procedure will eliminate too many false positive cows, in this situation the positive sera to Rose Bengal test are submitted to more definite confirmatory test such as complement fixation test (Radostits et. al., 2000).

2.10.3.3. Complement fixation test (CFT)

The CFT is a widely used and accepted confirmatory test. It is compels to perform, requiring good laboratory facilities and adequacy trained personnel. There are numerous variation of the CFT is use, but this test most conveniently carried out in microtitter plates. Either warm or cold fixation may be used for the incubation of the serum, antigen and complement: either at 37°C for 30 min or at 4°C for 14-18 hours. A number of factors affect the choice of the method: Anti complementary activity in serum samples for poor quality is more evident is cold fixation, while fixation at 37°C increases the frequency
and intensity of prozones and number of dilution must be tested for each sample (OIE manual, 2003).

2.10.3.4. Anti globulin Test (AGT) or Combs test

The test detects antibodies which don’t give rise to agglutination even if combined with antigen, because they are assumed to be in complete. It employs antispecies IgG to agglutinate antigen–antibody complex which is not visible by the ordinary SAT. It also detects blocking antibodies and therefore is used to diagnose chronic infection (Buxton and Fraser, 1977). On testing human and cattle material, the test was found to be more sensitive indicator of exposure to infection than SAT and CFT. However the test is laborious to perform, require anti–species globulins which are difficult to obtain by many laboratories and reacts positively in vaccinated animals (Morgan, 1989).

2.10.3.5. Indirect heamoagglutination test (IHAT)

The test was found useful for the diagnosis of brucellosis in animal and man. It uses LPS of *Br. abortus* or intracellular antigens and could be carried out as a tube or microtiter plate test (Corobl and Dan, 1937). The IHAT is highly sensitive but it is specificity was offset by difficulty of interpreting reactions produced at low dilution of sera.

2.10.3.6. Indirect enzyme linked immunosorbent assay (I-ELISA)

The I-ELISA was frist developed by (Carlson *et. al.*; 1976) for the diagnosis of human brucellosis. Since then, a large number of variations have been described. However the most common format uses smooth lipopolysaccharide (SLPs) antigen coated passively into polystyrene matrix.
The I-ELISA has several advantages over the conventional tests, the main advantage being that the antibodies to be detected reacts with the antigen without performing secondary function, such as agglutination, precipitation or activation of complement (Nielsen and Kwok, 1995). The OIE approved version of this test which uses purified smooth lipo polysaccharide (SLPs) as an antigen, serum diluted 1:50 and mouse monoclonal antibody specific for bovine IgG1 conjugated with horse reddish peroxidase (OIE manual, 2000). The same assay format may be used for testing milk. The I-ELISA assay may also be used for detection of caprine and ovine antibody resulting from *B. melitensis*, but, it failed to differentiate vaccinal antibody resulting from *B. abortus* strain 19 or *B. melitensis* Rev-1 vaccination from antibody induced by pathogenic strain (Nielsen and Gall, 1994). The ELISA detects antibodies, including IgG1 produced against other bacteria, such as *Yersina enterocolitica* 0:9 possible decreasing its specificity (Nielsen et al, 1996).

2.10.3.7. Competitive Elisa (C-Elisa)

The competitive Elisa using a MAb specific for one of the epitopes of the Brucella spp. O.chain polysaccharide has been shown to have higher specifically than the I-Elisa (Nielsen et al, 1995). This is accomplished by selecting a MAB that has higher affinity than cross reaction antibody. However it has been shown that the C-Elisa eliminated some but not all reaction due to cross-reacting bacteria (Weynant; et.al, 1997). The C-Elisa is also capable of eliminating most reaction due to residual antibody produced in response to vaccination with strain 19. The choice of MAB and it’s unique specificity and affinity will have a distinct influence on the diagnostic performance.
characteristic of the assay. The universal availability of the MAb of the hybridoma must also be considered with respect to international acceptance and wide spread use (OIE manual, 2003).

2.10.3.8. Milk ring test (MRT)

An adaptation of the agglutination test uses hematoxylin stained whole cell antigen added to milk (Huber and Nicoletti, 1986). If antibody is present in the milk, it will attach to milk fat globules via its FC portion. These antibodies will agglutinate with antigen and when the fat globules rise in the milk; a purple band will appear at the top of the milk. If no antibody is present, band will remain buff-colored. The test may be applied to pools of milk by increasing the amount of milk tested. While this is relatively insensitive test, subject to wrong interpretation caused by various milk conditions such as mastitis, colostrums and milk at the end of the lactating cycle, it is recommended by the OIE as screening test for bovine brucellosis (OIE manual 2000).

2.10.3.9. Fluorescence polarization assay (FPA)

The FPA was developed as a test that could be performed out side the diagnostic laboratory, allowing rapid and accurate diagnosis (Nielsen and Gall, 2001). The basic of the test is that a molecule in solution rotates randomly at rate inversely proportionally to its size. If the molecule is labeled with fluorescent marker and is examined by plane polarized light, small molecule will rotate through given angle faster than large one. The time of rotation may be measured using horizontal vertical measurement (Nielsen et al, 2000). For diagnosis of brucellosis, fluorescence polarization analyzer is used to obtain background measurement of fluorescence of diluted serum. Antigen
consisting of an OPS fragment, approximately 22KDa in size labeled with fluorescent ISO thyocyanate is added and incubated for two minute followed by final reading in the analyzer which automatically subtracts the background reading. The net result is presented in multipolarization unit. The FPA can be performed almost anywhere using a portable analyzer which receive power from a laptop computer, using serum, milk or EDTA anticoagulated blood (Nielsen and Gall 2001). The test is rugged, relatively inexpensive simple and very rapid.

2.10.3.10. Allergic skin test (AST)

It is routinely and officially used for the diagnosis of brucellosis in east European countries (Kolar, 1990). Kolar mentioned that the test could be used in farm animals but it was mainly intended for sheep, goat and pig. In cattle the test could be used to confirm or correct the result of serological test. In some countries it was found to be equivalent to serological test in cattle (Jerabek, 1962). AST is performed strictly into the skin. The sides of injection depend on the animal species. The test is specific and doesn't react to cross reacting organism (kolar, 1990). Some workers believe that the AST is more sensitive than the serological test (kolar and kolarova, 1955).

2.10.4. Molecular techniques
2.10.4.1. Polymerase chain reaction (PCR)

The techniques provide a promising option for the diagnosis of brucellosis. It is a potentially useful method that has been used in combination with labeled probe for the detection of Brucella species, from isolated bacteria or highly contaminated aborted tissue (Fekete et. al., 1990). Serum samples should be used preferentially over whole
blood for the molecular diagnosis in goat, cattle and human, and it was shown to be more sensitive than RBT and cultural techniques (leal-klevazasa et. al., 2000).

2.10. 5. Commonly used tests

The commonly used test in the diagnosis was rose Bengal test and the buffered antigen plate agglutination test (BPAT) (Morgan et. al., 1969). The rose Bengal test uses B. abortus S99 or S111.3 whole cell stained with crystal violet and brilliant green dyes. Both tests are standardized, simple to perform, inexpensive and considered suitable for screening individual animals (OIE manual 2000). The milk ring test is probably the most widely used test for the screening and monitoring of brucellosis in diary cattle (Alton et. al., 1988).

Although the sensitivity of the milk ring test is satisfactory, its specificity has been questioned when prevalence is low (Huber and Nicolleti, 1986).

2.11. Bovine brucellosis

Brucellosis in cattle is usually caused by biovars of Br. abortus. In some countries where cattle are kept in close association with sheep or goats infection can also be caused by B. melitensis. Occasionally, B. suis may cause an infection in the mammary gland of cattle, but it has not been reported to cause abortion (OIE manual, 2003). The disease is usually asymptomatic in non pregnant female, following infection with B. abortus or B. meletensis, pregnant adult females develop placentitis usually resulting in abortion between the fifth and ninth month of pregnancy. Even in the absence of abortion profuse excretion
of the organism occur in the placenta, fetal fluids and vaginal discharge (OIE manual, 2003). The mammary gland and associated lymph nodes may also be infected and the organism may be excreted in the milk. In acute infection, the organisms are present in most major lymph node. Adult male cattle may develop orchitis and brucellosis may cause infertility in both sexes.

Hygroma, usually involving leg joints, are common manifestation of brucellosis in some tropical countries and may be the only obvious indicator of infection, the hygroma fluid often contains brucella (OIE manual 2003).

2.12. Control and eradication of brucellosis

Brucellosis is a serious zoonosis disease, which is difficult to be cured because of the capacity of the organism to grow intracellularly, and because of the tremendous effect of the disease on economy and exportation. There is a justification to control or eradicate the disease in animals and hence in man (Musa, 1995). According to Musa 1995 there is two ways for control and prevention of brucellosis:-

- Vaccination of exposed herd or animals.
- Segregation of infected animals or herds from free ones and this is done by testing and slaughter or isolation of sero–positive animals.

2.13. Control of brucellosis in Sudan

Musa 1995 recommended that the first step toward control of brucellosis is education of all people concerned, this include nomads, animal owners, abattoir and butcher–house workers, and veterinary
supervision. The second step is to convince animal owners to get rid of their infected animals by selling them for slaughter under veterinary supervision. The most important control measure should be vaccination coupled with the step above. In Sudan vaccination programs were adapted to protect animals against brucella, even in farms in Khartoum with few exceptions.

2.14. Vaccination

2.14.1. *Br. abortus* strain 19 vaccine

It is the most widely used vaccine to prevent bovine brucellosis (Nicollet, 1990). The strain was able to induce protective immunity in cattle. Effectiveness fluctuates depending on variety of variables including age of vaccination, dose, route and prevalence of brucellosis in vaccinated herd (Nicoltti 1990). Strain 19 is an attenuated of smooth morphology normally unable to grow in the presence of erythritol (Jones *et al*; 1965). Although strain 19 is of low virulence for cattle, vaccination of pregnant cow can result in abortion (Mingle, 1941).

2.14.2. *Br. melitensis* Rev-1 vaccine

Rev-1 vaccine is alive, attenuated *Br. melitensis* strain derived from a virulent *Br. melitensis* isolate which become dependent on streptomycin for its growth, but lost this characteristic, although remaining streptomycin resistant, upon subculture (Elberg and Faunce, 1957). It stimulates protection against infection with *Br. melitensis* in sheep and goats and also protects rams against infection with *Br. ovis* (Alton *et al*., 1967, Fensterbank *et al*., 1982 and Alton, 1985). This vaccine is attenuated when compared with field strain but retain some
what virulence (Alton et al; 1967). Depending on the dose administrated during pregnancy, abortion will occur with variable frequency (Bardestein et al; 2002). Apparently in rams the vaccine is a virulent or of low virulence (Lantir and Fensterbank, 1985). Rev-1 is smooth organism, therefore induces positive serology which interferes with diagnosis. The use of Rev-1 in cattle has been investigated and results indicated that, it can give better protection than strain 19 (Garcia-Carrillo, 1980).

2.14.3. *Br. abortus* strain 45/20 vaccine

*Br. aborts* smooth strain 45/20 was isolated from cow in 1922 and a rough derivative was obtained after 20 passages in Guinea pigs. This derivative was able to protect Guinea pigs and cattle from brucella infection (McEwen and Priestly, 1938 and McEwen, 1940). Unfortunately, when used as a live vaccine, strain 45/20 was not stable and tended to revert to the smooth virulent form, thereby defeating the purpose of using rough strains, usually associated with attenuation and inability to conduce positive diagnostic serology (Edwards et al; 1945).

2.15. Hygienic measures

Hygienic measures in the farm should include clean cattle in the farm, separation of animals shortly before parturition, disposal of aborted fetus and placenta, disinfection of animals and their quarters. Animals should also be segregated after parturition or abortion until the vaginal secretion stop.
2.16. Treatment of brucellosis

A long term treatment with a high dose of oxytetracyclin had completely eliminated \textit{Br. melitensis} from naturally infected sheep (Radwan et al; 1987). In human many antimicrobial agents are used for the treatment of brucellosis, such as tetracycline, trimethoprim, sulfa methoxazole and streptomycin.
CHAPTER TWO
MATERIALS AND METHODS

1. Sterilization:
   Test tubes, bottles, flasks and pipettes were sterilized in the hot air over at 160°C for one hour.

2. Samples:
2.1. Type and source
   A total of 121 serum samples were collected from dairy cattle in AlHawata area, ElGadarif State during the period between March to April 2007. The examined animals comprised different breeds and different age groups (see table 2.1 and table 2.2).

3. Collection
   Blood samples were taken from cattle 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 7ml of blood was withdrawn using a labeled vacutainer. 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 to separate the serum. The serum was then separated from the whole blood by centrifugation, placed in sterile bijou bottles labled and stored frozen for months.
4. Serological test

4.1. Rose Bengal Plate Test (RBPT)

The antigen used in the RBPT was obtained from Central Veterinary Research Laboratory (CVRL), Soba. The sera and the antigen were brought to room temperature before testing.

The test was done as described by Alton et.al. (1975) by dispensing 0.03ml of each serum to be tested to an enamel plate and equal amount of RBPT antigen was added to each serum sample and both were mixed together, rocked by hand for four minutes, after which the test was immediately read. 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.

Positive results to RBPT were classified into five categories according to Alton et. al. (1975):

1. Weak positive: When very weak fine agglutination occurred, this could be hardly seen by necked eyes.
2. Positive: when agglutination was fairly visible
3. Positive with ring formation: When the agglutination forming a ring.
4. Strong positive: where there was a granular agglutination
5. Very strong positive: where the agglutination was very rapid and large clumps occurred, leaving only clear fluid.
4.2. Serum Agglutination Test (SAT):

The test was done according to Alton et. al. (1975).

Materials:

- Antigen: *Br. Abortus* strain 99 is usually used in preparing the antigen. Other smooth strains of *Br. abortus* and *Br. suis* may serve equally well. But only strains of proved agglutinating ability should be used. This strain is propagated by culturing in Potato agar.

- Glass or plastic tube (8mmx50mm) with rim and metal agglutination boxes for carrying the tubes.

- Automatic pipette and tips

- Phenol-saline

- Tested serum sample.

Methods:

- 0.8 ml of phenolsaline was placed in the first tube and 0.5ml in each succeeding tube.

- 0.2ml of the serum under test was transferred to the first tube and mixed thoroughly with the phenolsaline already there.

- 0.5 ml of the mixture was carried soon over the second tube. This process is continued until the last tube, from which after mixing, 0.5 ml of dilution was discarded. This process of doubling
dilution results in 0.5ml of dilutions 1:5, 1:10, 1:20 and so on in each tube.
- To each tube 0.5ml of antigen was then added at the recommended dilution and the contents of the tube were thoroughly mixed, thus giving final serum dilution of 1:10, 1:20 etc…
- The tubes were then incubated at 37°C for 20 hours before the results are read.

**Interpretation of results:**

The degree of agglutination was assessed by the amount of clearing that has taken place in the tube as compared with a standard tube. The tubes were examined, without being shaken, against a black background, with a source of light coming from above and behind the tubes. Complete agglutination and sedimentation with water-clear supernatant was recorded as ++++, nearly complete agglutination and 75% clearing as +++, marked agglutination and 50% clearly as ++, some sedimentation and 25% clearing as +, and no clearing as standards were prepared at the time the tests were done and incubated with them. The antigen was diluted by mixing 2ml of antigen, diluted as for the test, with 2ml of phenol-saline.

**4.3. C-ELISA**

The test was carried out as described by (Veterinary laboratory agency).
Materials:

4.3.1. Kit content:
   a. Plates: Plates pre-coated with *B. melitensis* LPS antigen.
   c. Wash solution: Na₂HPO₄ + Tween 20
   d. Conjugate: As supplied (Store at -20°C)
   e. Chromogen: OPD tablets (toxic)
   f. Substrate: urea hydrogen peroxide tablets
   g. Stopping solution: citric acid
   h. Control: positive serum and Negative serum

4.3.2. Equipment Required
   Microtitre plate reader with 450nm filter
   Single and multichannel variable volume pipettes
   Disposable tips for the above
   Reagent troughs for multichannel pipetting
   10 litre container for wash fluid
   4°C ± 3°C refrigerator
   Rotary shaker, capable 160 Revs/Min (or a 37°C ± 3°C incubator)
   Microtitre plate shaker
   Sterile distilled or deionised water
   Bottles, tubes and beakers for storage of sera and reagents
   Absorbent paper towels
   Freezer for storage of conjugate
Notes:

The microtitre plate reader was not essential as an assessment of the results can be performed visually.

Method

1. The conjugate solution was prepared immediately and diluted to working strength with diluting buffer according to instructions on the ampoule label.
2. 20µl of each test serum was added per well and columns 11 and 12 were left for controls.
3. 20µl of the negative control was added to wells A11, A12, B11, B12, C11 and C12.
4. 20µl of the positive control was added to wells F11, F12, G11, G12, H11 and H12.
5. The remaining wells (had no serum) were act as the conjugate controls.
6. Immediately 100µl of the prepared conjugate solution was dispensed. This gave a final serum dilution of 1/6.
7. The plate was then vigorously shaken (on the microtitre plate shaker) for 2 minutes in order to mix the serum and conjugate solution. The plate was covered with the lid and incubated at room temperature (21°C ± 6°C) for 30 minutes on a rotary shaker, at 160 revs/min.
8. The contents of the plate was shaken out and the plate was rinsed 5 times with washing solution and then thoroughly dried tapping on absorbent paper towel.
9. The microplate reader was switched on and allowed unit to stabilize for 10 minutes.
10. Immediately before use the substrate and chromogen solution were prepared by dissolving one tablet of urea H$_2$O$_2$ in 12 ml of distilled water. When dissolved the OPD tablet was added and mixed thoroughly. This took a few minutes; the use of a magnetic stirrer greatly increased the speed with which it dissolved. 100µl of this solution was added to all wells. (This solution was not stored).

11. The plate was left at room temperature for a minimum of 10 minutes and a maximum of 15 minutes.

12. The reaction was slowed by adding 100µl of stopping solution to all wells.

13. Condensation from the bottom of the plate was recovered with absorbent paper towel. Read plate at 450nm.

If a microtitre plate reader was not available a visual inspection of the plate was used to determine whether a sample was positive or negative.

**Analysis of Results:**

The lack of colour development indicated that the sample tested was positive. A positive/negative cut-off was calculated as 60% of the mean of the optical density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to or below this value was regarded as positive.

**Trouble Shooting**

**No colour at all even after 15 minutes incubation**

- No hydrogen peroxide was added to OPD solution
- OPD made up incorrectly
- Conjugate not working at the dilution used in the test
- No activity in the hydrogen peroxide
- Hydrogen peroxide at wrong dilution
- Conjugate was not dispensed onto plates

**Colour develops too slowly**
- Hydrogen peroxide too weak
- Conjugate too weak

**Colour develops too quickly**
- Conjugate made up incorrectly
- Poor quality distilled water
- Substrate/chromogen is contaminated

**Colour all over plate**
- Conjugate made up incorrectly
- Poor washing
- Substrate/chromogen is contaminated
- Diluting buffer made up incorrectly

**Patchy or poor colour**
- Poor pipetting or washing
- Poor mixing of reagents
- Dirty glassware
- Poor quality distilled water

**Evaluation of test results:**
- The test results of each plate were evaluated by checking the following values.
  
  Binding Ratio = \[
  \text{Mean of 6 negative control wells} \quad \text{Mean of 6 positive control wells}
  \]
- The binding ratio must be greater than 10.
• The mean OD of six negative control wells must be greater than 0.7.
• The mean OD of six positive control wells must be less than 0.1.
• The mean OD of the four conjugate control wells must be greater than 0.7.
• Any plate tests results which did not comply with the above values were rejected and samples re-examined.
Table (2.1):

Age group

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than or equal to 8 years</td>
<td>75</td>
<td>62.0</td>
</tr>
<tr>
<td>More than 8 years less than 12 years</td>
<td>32</td>
<td>26.4</td>
</tr>
<tr>
<td>More than 12 years</td>
<td>14</td>
<td>11.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>121</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Table (2.2)

Breeds of animals

<table>
<thead>
<tr>
<th>Animal breed</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenana</td>
<td>66</td>
<td>54.5</td>
</tr>
<tr>
<td>Rufaa</td>
<td>21</td>
<td>17.4</td>
</tr>
<tr>
<td>Sharig</td>
<td>31</td>
<td>25.6</td>
</tr>
<tr>
<td>Cross</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>121</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
CHAPTER THREE
RESULTS

A total of 121 serum samples from cattle were investigated for antibodies against Brucellosis using different serological tests.

The relation of results to breed, history of abortion and age was studied.

3.1. Serological investigation:

Results are shown in tables 3.1, 3.2 & 3.3. All serum samples were investigated using RBPT and cELISA. 19 samples (15.7%) were found positive with RBPT (table 3.1) and 20 samples (16.5%) were found positive with cELISA. The result of the two tests was identical except in one sample which was found positive with cELISA (table 3.3).

Only positive samples were subjected to further investigation using SAT. Results are shown in tables 3.4 and 3.5. All samples were found positive including the sample which gave different reading with RBPT and c-ELISA they had antibody titre ranged between 17-1280.
Table 3.1.: Result of Rose Bengal test

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>102</td>
<td>84.29752</td>
<td>84.29752</td>
<td>84.29752</td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>15.70248</td>
<td>15.70248</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2.: Result of ELISA

<table>
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<tr>
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<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>101</td>
<td>83.47107</td>
<td>83.47107</td>
<td>83.47107</td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>16.52893</td>
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<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>100</td>
<td>100</td>
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</table>

Table 3.3.: Result of cross-tabulation of ELISA and Rose Bengal

<table>
<thead>
<tr>
<th>Result of ELISA</th>
<th>Result of Rose Bengal test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
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<td>101</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>121</td>
</tr>
</tbody>
</table>
Table 3.4.: Antibody titre for brucellosis using SAT

<table>
<thead>
<tr>
<th>Valid</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>1</td>
<td>0.826446</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>1.652893</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>67</td>
<td>1</td>
<td>0.826446</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>80</td>
<td>4</td>
<td>3.305785</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>106</td>
<td>2</td>
<td>1.652893</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>160</td>
<td>2</td>
<td>1.652893</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>186</td>
<td>1</td>
<td>0.826446</td>
<td>5</td>
<td>65</td>
</tr>
<tr>
<td>212</td>
<td>1</td>
<td>0.826446</td>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td>320</td>
<td>1</td>
<td>0.826446</td>
<td>5</td>
<td>75</td>
</tr>
<tr>
<td>372</td>
<td>2</td>
<td>1.652893</td>
<td>10</td>
<td>85</td>
</tr>
<tr>
<td>640</td>
<td>1</td>
<td>0.826446</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>744</td>
<td>1</td>
<td>0.826446</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>1280</td>
<td>1</td>
<td>0.826446</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>16.52893</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

Missing System: 101  83.47107

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number of samples</th>
<th>Range of titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenana</td>
<td>11</td>
<td>17-1280</td>
</tr>
<tr>
<td>Rufaa</td>
<td>4</td>
<td>67-744</td>
</tr>
<tr>
<td>Sharig</td>
<td>4</td>
<td>80-640</td>
</tr>
</tbody>
</table>
3.2. Effect of age:

Results are shown in tables 3.6. and 3.7 and figure 3.1. The two tests showed that 17.3% of animals $\geq$ 8 years old in contrast to 21.4% of animals $\geq$ 12 years old were found positive.

The highest antibody titre was detected in animals $\geq$ 8 years old. In addition most of animals with low antibody titre were also detected in animals $\geq$ 8 years old (Figure 3.2).

Table 3.6.: Result of ELISA in the different age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Result of the ELISA</th>
<th>Count</th>
<th>% within Age group</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than or equal to 8 Years</td>
<td>Count</td>
<td>62</td>
<td>82.7%</td>
<td>13</td>
<td>17.3%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>% within Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 8 Years less than 12 Years</td>
<td>Count</td>
<td>28</td>
<td>87.5%</td>
<td>4</td>
<td>12.5%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>% within Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 12 Years</td>
<td>Count</td>
<td>11</td>
<td>78.6%</td>
<td>3</td>
<td>21.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>% within Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>101</td>
<td>83.5%</td>
<td>20</td>
<td>16.5%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>% within Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.7.: Result of Rose Bengal test in different age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Result of Rose bengal test</th>
<th>Count</th>
<th>% within Age group</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than or equal to 8 Years</td>
<td>Count</td>
<td>82</td>
<td>82.7%</td>
<td>13</td>
<td>17.3%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>% within Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 8 Years less than 12 Years</td>
<td>Count</td>
<td>28</td>
<td>87.5%</td>
<td>4</td>
<td>12.5%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>% within Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 12 Years</td>
<td>Count</td>
<td>12</td>
<td>85.7%</td>
<td>2</td>
<td>14.3%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>% within Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>102</td>
<td>84.3%</td>
<td>19</td>
<td>15.7%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>% within Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.1.: Result of ELISA and Rose Bengal in different age groups

<table>
<thead>
<tr>
<th></th>
<th>Less than or equal to 8 Years</th>
<th>More than 8 Years less than 12 Years</th>
<th>More than 12 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>17.33%</td>
<td>12.50%</td>
<td>21.43%</td>
</tr>
<tr>
<td>Rose Bengal</td>
<td>17.33%</td>
<td>12.50%</td>
<td>14.29%</td>
</tr>
</tbody>
</table>

Figure 3.2.: The titer of SAT in different age groups

A: Distribution of antibody titre in animals less than or equal to 8 Years.
B: Distribution of antibody titre in animals more than 8 Years less than or equal to 12 Years.
C: Distribution of antibody titre in animals more than 12 Years.
3.3. Effect of breed:

Results are shown in tables 3.8. and 3.9. Among the investigated animals, the cross animals were the most frequently affected group (33.3%).

Table 3.8.: Result of Rose Bengal test in different breeds

<table>
<thead>
<tr>
<th>Breed</th>
<th>Count</th>
<th>% within Breed</th>
<th>Result of Rose Bengal test</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenana</td>
<td>56</td>
<td>64.8%</td>
<td>Negative</td>
<td>56</td>
<td>10</td>
</tr>
<tr>
<td>Rufaa</td>
<td>17</td>
<td>81.0%</td>
<td>Negative</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Sharig</td>
<td>27</td>
<td>87.1%</td>
<td>Negative</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>Cross</td>
<td>2</td>
<td>66.7%</td>
<td>Negative</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>84.3%</td>
<td>Negative</td>
<td>102</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 3.9.: Result of ELISA test in different breeds

<table>
<thead>
<tr>
<th>Breed</th>
<th>Count</th>
<th>% within Breed</th>
<th>Result of the ELISA</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenana</td>
<td>55</td>
<td>83.3%</td>
<td>Negative</td>
<td>55</td>
<td>11</td>
</tr>
<tr>
<td>Rufaa</td>
<td>17</td>
<td>81.0%</td>
<td>Negative</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Sharig</td>
<td>27</td>
<td>87.1%</td>
<td>Negative</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>Cross</td>
<td>2</td>
<td>66.7%</td>
<td>Negative</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>83.5%</td>
<td>Negative</td>
<td>101</td>
<td>20</td>
</tr>
</tbody>
</table>
3.4. Relation between Brucellosis and history of abortion:

Results are shown in tables 3.10 and 3.11 and figure 3.3. Among the investigated animals Seventeen cows had a history of abortion. Only 5 of which were found positive for Brucellosis.

Table 3.10.: The result of Rose Bengal in animal with and without history of abortion

<table>
<thead>
<tr>
<th>History of Abortion</th>
<th>Count</th>
<th>% within History of Abortion</th>
<th>Result of Rose bengal test</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without</td>
<td>90</td>
<td>86.5%</td>
<td>Negative</td>
<td></td>
<td>14</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
<td>13.5%</td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>12</td>
<td>70.6%</td>
<td>Negative</td>
<td></td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
<td>29.4%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>84.3%</td>
<td>Negative</td>
<td></td>
<td>19</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
<td>15.7%</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.11.: The result of ELISA in animal with and without history of abortion

<table>
<thead>
<tr>
<th>History of Abortion</th>
<th>Count</th>
<th>% within History of Abortion</th>
<th>Result of the ELISA</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without</td>
<td>89</td>
<td>85.6%</td>
<td>Negative</td>
<td></td>
<td>15</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
<td>14.4%</td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>12</td>
<td>70.6%</td>
<td>Negative</td>
<td></td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
<td>29.4%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>83.5%</td>
<td>Negative</td>
<td></td>
<td>20</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
<td>16.5%</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.3. The result of ELISA and Rose pBengal in animal with history of abortion
The existence of brucellosis in the Sudan for a long time together with lack of proper eradication programme resulted in a wide spread of the disease.

In the current study the presence of the disease in ElHawata area, ElGadarif State, Eastern Sudan was studied for the first time. Twenty samples (16.5%) out of 121 bovine sera were found positive for brucellosis. This finding was comparable to other studies, Daffalla (1962), Fayza (1990) and Musa (1995) report that prevalence rate for brucellosis was 10.7% (in ElGazira), 15.73% (Khartoum State) and 13.7% (in Darfur States) respectively. However, it was different from the finding of Abdalla (1966) who found that the overall prevalence rates of brucellosis in cattle in Northern Sudan was 3%, that may due to small animal population and the environmental condition in Northern Sudan.

Brucellosis may spread in ElHawata area, due to many reasons which may include mixing of different animal species together (goats, sheep & cattle), Nomads know little about the disease, they screen cattle for brucellosis for export and retain the positive ones within their herd. Lack of control measures whether by isolation of infected animals, vaccination or stamping out of infected cattle was also thought to be responsible for the high rates of infection. The nomadic nature of the husbandry method and continuous movement of cattle from one locality to another exposing them to brucella infection.
Serum samples were screened for antibodies against Brucellosis using RBPT. It was the only test used for routine diagnosis of brucellosis in Sudan. The result was confirmed with c-ELISA & further with SAT. 99.2% of results agreed with that of c-ELISA indicating that the test was sensitive. However it may gave false –ve result which may lead to spread of the disease. In another study, Mukhtar (2007) reported that c-ELISA was more sensitive than RBPT.

The positive samples with c-ELISA was confirmed with SAT and this may indicate that one of these tests is enough to confirm the result of screening test.

Antibodies against Brucellosis were detected in all animal groups. However the higher percentage of affected animals were observed in cows ≥ 12 years old. This result was similar to that of Raias (2004) who found that the old animal is susceptible to the disease more than young one. This could be explained by the fact that once an animal is infected, it can not be treated. In addition infected older animals may not abort (i.e showing no clinical signs of the disease) and accordingly remain hidden in the herd (carriers) and a source of infection to others. The highest titre was detected in animals ≥ 8 years old & this may probably indicate recent infection.

Four different cow breeds were included in the study and they represent breeds in ElHawata area. The highest percentage of infected animals was detected in cross breed although the number of samples from this breed was small. This may be attributed to the fact that local breeds are generally resistant to diseases in the tropics compared to foreign ones.
Only 29.4% of cows with history of abortion were found positive for brucellosis. This finding indicated that the diagnosis of the disease should not be revealed only on cases of abortion and usually confirmatory tests are needed to a certain the presence of the disease. And the abortion may be due to miss use of drugs and infection with other microorganisms which cause abortion.

**Conclusion:**

It was concluded from this study that bovine Brucellosis was present in El Hawata area, and affects different age and breed groups.

**Recommendation:**

**It is recommended that:**

1. The distribution of the disease in all Sudan States should be studied.
2. Proper control measure should be followed to reduce the infection rate.
3. Education of people like animal owners, nomads and abattoir workers is essential to increase their awareness to avoid infection and prevent their animals from the disease and environment from contamination.
CHAPTER FIVE

References


Cunningham (1977a,b); Cited by Nicoleffc (1980).


Delvecchio, V.G.; V. Kapatral, P.Elzer, G. Patra, C.V. Mujer. 2002

“The genome of brucella mettensis microbial (587-592)


Perry, M.B.; Budle, D.R. and Cherwonongrodzky, J.W. (1986). The structure and serology of the A and M antigen of *Br. abortus* and *brucella melitensis* and the structure of


