STUDIES ON ANIMAL BRUCELLOSIS IN RED SEA STATE SUDAN

Hatim Mohamed Mohamed Ahamed
B. V. Sc. 1982 University Of Zagazig Egypt

A Thesis Submitted To The University Of Khartoum In Partial Fulfillment Of The Degree Of Master Of Veterinary Science

Under The Supervision Of:
Dr. Tawfig Eltigani Mohamed
Faculty Of Veterinary Medicine,
University Of Khartoum

Department Of Preventive Medicine And Public Health
Faculty Of Veterinary Medicine
University Of Khartoum
June 2004
DEDICATION

To the soul of my father,

To my mother,

Brothers

Sisters

And wife
I would like to thank my supervisor Dr. Tawfig ElTigani Mohammed, Department of Preventive Medicine, University of Khartoum for his close supervision and guidance during the course of study. I wish to express my faithful acknowledgement to the Director of Regional Research Laboratory, Port Sudan, Dr. Adil Ali for his great help during this work. My gratitude is extended to professor Ali Mohamed Abd ElMajid Director of Central Veterinary Research Laboratory for allowing me to do this work. Also I would like to thank the technicians and laborers at Veterinary Quarantine, Port Sudan, mainly Hassan Omer for their help. I am also grateful to Miss Tahani Hassan Ali for her care in typing the thesis.
The work described in this thesis was carried out in the Department of Preventive Medicine and Veterinary Public health, Faculty of Veterinary Medicine, University of Khartoum under the supervision of Dr. Tawfig ElTigani Mhamed.
A serological study for brucellosis was carried out to determine the magnitude & spread of the disease in animals in Red Sea State. Milk Ring test and Rose Bengal Plate Test were used for the diagnosis of the disease.

A total of 4566 sera were collected, 210 from cattle, 356 from camel's, 2000 from sheep; and 2000 samples from goats, and a total of 165 milk samples were collected, 65 from cattle, 50 from sheep. 50 samples from goats. Serum samples were tested with RBPT and the milk samples were tested with MRT.

The prevalence rate was, 11.9%, 19.1%, 0.3% and 0.45% in cattle, camels, sheep and goats, respectively.

Difference between percentage of infected males and females in camels, sheep and goats were found to be negligible, but in cattle the percentage of infected females was higher (22.36%) than in males (5.9%). In cattle the prevalence rate in relation to age was found to be increased by age, and in camels was high in animals 3-10 years old (28.2%), and in sheep and goats was high in adult animals. The infection rate was found to be high in diary farms (22.2%).
ملخص الطرودة

工业大学 / حجم المعارف وبالغ / البقر، البقر، الخيل، في دراسات أجرت لقد وامتحان الصحي بمنع ذلك، انتشر في البروسيليا مرض لتشخيص الحلقية.

المجموع تم لعدد 4566 سيم، عينة 210 البقر، عينة 356، الجمال عينة 2000 عينة، والضأن عينة 2000 المئذن، يتمام他们都 تم الصحة بمنع ذلك، وامتحان النمر في سيرم، عينة 65 الحلقية عينة 165، ديم، يتمام 3000 بلوس، وامتحان النمر في 50، واستخدام النمر في 50. 

كما كان بمرض الاصابة نسبة في 19.1%، في 0.3%، في 0.45%، بالنسبة للبن، نسبة بمرشد في 11.9%， في 19.1% النسبة، بالنسبة للبن، بمرشد في 0.45% النسبة، بالنسبة للبن، بمرشد في 50، استخدم النمر في 50، نسبة في 5.9%， نسبة، وامتحان النمر في 22.36% النسبة، في 22.36% النسبة، وكب، النسبة نقص في 28.2% النسبة، في 10-3 أمراض وấجة النسبة، (22.2%) لامتحان إجراء النسبة نقص في 10.3، وامتحان النسبة، (22.2)
CONTENTS

Dedication ........................................................................................................... i
Acknowledgment ................................................................................................ ii
Preface .................................................................................................................... iii
Abstract .................................................................................................................. iv
Arabic abstract ....................................................................................................... v
Contents ................................................................................................................. vi
List of tables .......................................................................................................... viii
Introduction .......................................................................................................... 1

Chapter one: Literature review
1.1 Historical background .................................................................................. 3
  1.1.1 History of brucella infection in animals ............................................... 3
  1.1.2 History of brucella infection in man ...................................................... 4
1.2 The genus brucella ....................................................................................... 5
  1.2.1 Morphology ......................................................................................... 6
  1.2.2 Culture and growth characteristics ..................................................... 6
  1.2.3 Biochemistry ...................................................................................... 8
  1.2.4 Antigenic characteristics .................................................................. 9
  1.2.5 Susceptibility to phages ................................................................... 10
  1.2.6 Susceptibility to dyes and antibiotics .............................................. 10
1.3 Taxonomy of the Genus brucella ............................................................... 11
1.4 Brucellosis ..................................................................................................... 19
  1.4.1 Definition ........................................................................................... 9
  1.4.2 Aetiology .......................................................................................... 19
  1.4.3 Transmission of the disease .............................................................. 20
    1.4.3.1 Routes of excretion and contagious material .......................... 20
    1.4.3.2 Mode of infection .................................................................... 20
    1.4.3.3 Survival of brucella in the environment .................................. 21
  1.4.4 Pathogenesis and immune response ................................................. 25
  1.4.5 Diagnosis .......................................................................................... 26
    1.4.5.1 Direct smear ............................................................................. 26
    1.4.5.2 Culture of samples to isolate the causative agent ................... 26
    1.4.5.3 Guinea pig inoculation ............................................................ 27
    1.4.5.4 Other method for agent detection ........................................... 27
    1.4.5.5 Serological tests ....................................................................... 28
      1.4.5.5.1 Rose Bengal Plate Test ..................................................... 28
      1.4.5.5.2 Serum agglutination test .................................................... 30
      1.4.5.5.3 Complement Fixation test ............................................... 30
      1.4.5.5.4 Enzyme-Linked immunosorbent assay ......................... 31
1.4.5.5 Antiglobulin (Coombs’) test ----------------------- 32
1.4.5.6 Tests for detecting antibodies in milk---------- 33
  1.4.5.6.1 Milk Ring Test ------------------------------- 33
  1.4.5.6.2 Whey Agglutination Test ---------------------- 34
1.4.5.7 Allergic Skin Test (AST) ----------------------- 34
1.4.5.8 Control and Eradication of brucellosis--------- 35
1.4.5.9 Treatment of brucellosis ----------------------- 38
1.4.5.10 Vaccination ---------------------------------- 38
  1.4.5.10.1 Brucella abortus S19 vaccine ------------- 38
  1.4.5.10.2 Brucella abortus 45/20 vaccine --------- 40
  1.4.5.10.3 Brucella melitensis Rev.1 vaccine ------ 40
  1.4.5.10.4 H38 vaccine ----------------------------- 41
  1.4.5.10.5 Br. Suis S2 ------------------------------ 41
  1.4.5.10.6 Br. abortus RB51 ------------------------ 42
1.4.5.11 The situation of brucellosis ------------------ 42
  1.4.5.11.1 The situation of brucellosis worldwide -- 42
  1.4.5.11.2 The situation of brucellosis in the Sudan 43
    1.4.5.11.3 The situation of Bovine Brucellosis in the Sudan 44
  1.4.5.11.4 The situation of camels brucellosis in the Sudan 45
  1.4.5.11.5 The situation of small ruminants brucellosis in the Sudan 46

Chapter Two: Material and Methods
2.1 Sterilization ---------------------------------------- 47
2.2 Sources and type of samples ----------------------- 47
2.3 Collection of samples ----------------------------- 48
  2.3.1 Serum samples --------------------------------- 48
  2.3.2 Milk samples ---------------------------------- 48
2.4 Serological Test ------------------------------------ 49
  2.4.1 RBPT ------------------------------------------ 49
  2.4.2 MRT ------------------------------------------- 50

Chapter Three: Results
3.1 Cattle --------------------------------------------- 51
3.2 Camels -------------------------------------------- 51
3.3 Sheep --------------------------------------------- 51
3.4 Goats --------------------------------------------- 52

Chapter Four: Discussion------------------------------- 57
Recommendation ---------------------------------------- 64
References --------------------------------------------- 65
LIST OF TABLES

No. page

1. Table 1 Differential characteristics of brucella from some other gram-negative bacteria .........................................................14
2. Table 2: Differential characteristics of brucella phase ...............15
3. Table 3: Biovar differentiation of the species of the brucella according to Alton et al.1988. .........................................................16
4. Table 4: Classification of the genus brucella according to Corbel 1990. ...................................................................................17
5. Table 5: Biovar differentiation of brucella species involved in sheep and goats brucellosis.................................................................18
6. Table 6: Studies on brucella survival time in the environment ....23
7. Table 7: Studies on brucella survival time in diary products ..........24
8. Tables (8&9): Prevalence rates of brucellosis in cattle in relation to sex and age. .........................................................................................53
9. Tables (10&11): Prevalence rates for brucellosis in camels in relation to sex and age .................................................................................54
10. Tables (12&13): Prevalence rates for brucellosis in sheep according to sex and age ................................................................................55
11. Tables (14&15): Prevalence rate for brucellosis in goats according to sex and age ...............................................................................56
INTRODUCTION

Brucellosis is primarily a disease of animals which can be transmitted to man either directly or indirectly (Anthropozoonosis), and it continues to be a zoonosis of worldwide public health and economic importance. The causative bacterium was named in honour of Sir David Bruce the discoverer of *Br. Melitensis*. The hallmarks of animals' brucellosis are abortion, infertility and reproductive failure (Philip, 2003).

The genus *brucella* contain six recognized species including: *Br. Melitensis* the main causative agent of brucellosis in sheep and goat, but the infection also occurs in cattle, camels and wild animals (Alton, Jones and Pietz, 1975; Elberg, 1983), *Br. Abortus* the cause of contagious abortion in cattle, can also infect bison, buffalo, camels, horses, chamois, dogs, fox and water buck, while infection of sheep and goats and pigs is rare (Eiberg, 1983). Although *Br. Suis* normally infect swine, infection of other animals such as dogs, caribon, reindeer and rabbits has also been reported (Elberg, 1983). *Br. Cains* causes a highly infectious form of brucellosis in dogs, *Brucella neotoma* infects the desert rat (*Neotoma lepida*), and *Br.ovis* infects rams.

Prior to the use of the inclusive term brucellosis, the disease in cattle was known by many names. These are 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 synonymously used for undulant fever (Carpenter and Hubbert, 1963).

The work undertaken here was carried out to survey the presence of brucella antibodies in milk and sera from domestic farm animals in Red Sea area.

CHAPTER ONE
LITERATURE REVIEW

1.1 Historical background
1.1.1 History of brucella infection in animals

Carpenter and Hubbert (1963) stated that in central Europe where abortion was rampant in cattle, the disease was considered infectious even in the sixteenth century. In a book “the Complete Farmer” it was stated that: “the foetus and forestal membrane were considered contagious and when it happens the abortion should be immediately buried and the cow kept as widely apart as possible from the herd.” (Cited by Bang, 1897). This proves that contagious abortion was a worry to cattle breeders for along time. As a result, Nocard was commissioned to study the epizootic abortion which prevailed among cattle at that time (Bang, 1897). In this investigation, Nocard described two morphologically different microbes in uterine exudates of an aborted cow and distinguished them as micrococci and thick bacilli. The investigator cultivated the two microbes in gelatin peptone or bouillon and obtained cultures similar to those he had described from uterine exudates. Unfortunately, Nocard could not reproduce the disease with either of the two isolate. Taking advantage of Nocard’s inconclusive work, Bang (1897) continued the investigations. He prepared cover glass preparations from the yellowish exudates of the allantoic fluid, stained them with methylene blue and demonstrated small bacteria in pure form, some of which were found intracellular. He then concluded that the epizootic abortion was a specific uterine catarrh caused by definite bacterium. Furthermore, Bang recovered the bacterium in a pure form using serum-gelatin agar and could always demonstrate and cultivate typical abortion bacilli from
different abortion material. The investigator noticed that the organism remained viable in the uterus and could cause abortion for the second time in previously aborted cows. He was able to reproduce the disease by injection of pure cultures into the vaginas of pregnant cows, thus proving that the organism he discovered was the cause of epizootic abortion. Further experiments on ewes and mares proved the occurrence of the disease in those species. He had also noticed that, goats kept in contact with aborted cows also aborted. It in recognition of Bang’s work that brucellosis is often named Bang’s disease.

1.1.2. History of brucella infection in human

Malta fever was earlier known by other names such as adenotyphoid, intermittent typhoid, gastric and bilious remittent fever (Bruce, 1887). The disease had a wide distribution in the Mediterranean area. Bruce described it as: Having along duration, a fever which often runs high and continuous, remittent and intermittent in type. The patient suffer rise in temperature, profuse sweating and the spleen is often enlarged. There are always rheumatic or neuralgic pains, Joints involvement and orhitis. " David Bruce isolates the etiologic agent of the fever in (1887) from the spleen of four fatal human cases on the Island of Malta. Bruce named the bacterium Micrococcus melitensis and the same worker renamed the species a Brucella melitensis in 1887 to honor the work of Bruce (Smith, 1979). The disease was recognized as a zoonotic by Zammit (1905) who proved that man acquired Malta fever by consumption of infected goats' milk. Mohler and Traum (1911) isolate *Br. Abortus* from a guinea pg inoculated with a tonsil material from
a child, and that was the first instance in which the organism was isolated from a human source. In Traum (1914) isolated *Br. Suis* from aborted swine. Kaeer (1924) was able to isolate *Br. Suis* from blood of a patient with undulant fever. The disease was thus identified as an animal disease transmissible to man. Bevan (1921) in Rhodesia demonstrated by culture and serological tests that *Br. Abortus* could cause "undulant fever" in man.

Even (1918) pointed out that Micrococcus melitensis described by Bruce and *Bacillus abortus* isolated by Bang were morphologically and antigenically similar. Later, such organisms were grouped in one genus; Brucella. Meyer was the first to suggest that the generic name Brucella in the family Bacteriaceae (Young Corbel, 1989).

### 1.2 The genus *brucella*

Members of the genus *brucella* are parasite of animals which may be transmitted to humans. These bacteria are characterized by their intracellular existence and their ability to infect all animal tissues where they cause infectious processes including contagious abortion in cows, goats and hogs, and undulant fever in man (Finegold and Martin, 1982; Finegold and Baron, 1986).

#### 1.2.1 Morphology

The genus brucella comprises a small group of closely related gram-negative bacteria currently classified as small, non-motile, non-spore forming, gram-negative cocci, coccobacilli or short rods, 0.5-0.7 um by 0.5-1 um, arranged singly, and les commonly in pairs or short chains. Members of the genus brucella
do not show bipolar staining or produce capsules. They do not form flagella or pili. They are not truly* acid-fast, but resist decolouration by weak acids, thus stain red by the stam's modification of Ziehl-Neelsen method, which is sometimes used for the microscopic diagnosis of brucellosis from smears of slide or liquid specimens.


1.2.2 Culture and growth characteristics

Brucella members are aerobic bacteria showing no growth under strictly anaerobic conditions (Brinley-Morgan and McCullough 1974; Corbel and Hendry, 1983). Some strains require an atmosphere containing 5-10% carbon dioxide (co₂) added for growth, especially on primary isolation. The optimum pH for growth varies from 6.6-7.4, and culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature is 36-38 degree centigrade, but most strains can grow between 20-40 degree centigrade. Brucellae require multiple amino-acids, biotin, thiamin and nicotinamide. The growth is improved by serum or blood, but haemin (V-factor) are not required. The growth of most brucella strain is inhibited on media containing bile salts, tellurite or selenite.

Growth is usually poor in liquid media unless culture is vigorously agitated. Growth in static liquid media favors dissociation of smooth-phase cultures to non-smooth forms. Continuous and vigorous aeration will prevent this,
provided a neutral pH is maintained. In semi-solid media, co₂-independent brucella strains produce a uniform turbidity from the surface down to a depth of a few millimeters, while cultures of co₂-requiring strains produce a disk of growth a few millimeters below the surface of the medium. On suitable solid media brucella colonies are visible after 2 days incubation. After four days incubation, brucella colonies are round, 1-2 mm in diameter, with smooth (S) margins, translucent and a pale honey colour when plates viewed in the day light through a transparent medium. When viewed from above, colonies appear convex and pearly white. Later, colonies become larger and slightly darker.

Smooth brucella cultures have a tendency to undergo variation during growth, especially with subcultures, and dissociate to rough (R) forms, and sometimes mucoid (M) forms. Colonies are then much less transparent and a more granular, dull surface (R) or sticky glutinous texture (M), and range in colour from matt white to brown in reflected or transmitted light. Intermediate (I) forms between S,R and M forms may occur in cultures undergoing dissociation to the non-smooth state. Changes in the colonial morphology are generally associated with changes in virulence, serological properties and phage sensitivity (Scientific Committee on Animal Health and Animal Welfare, July 2001).

1.2.3 Biochemistry

The metabolism of brucella species is oxidative and usually shows little fermentative action on carbohydrates in conventional media. Many strains require supplementary (5-10%) carbon dioxide for growth especially on primary isolation.
Although brucella neotomae, *Br. ovis* and some strains of *Br. Abortus* are catalase positive and oxidase negative, other brucellae are catalase positive and oxidase positive. The bacterium reduces nitrates, produces hydrogen sulfide and hydrolyzes urea to variable extent. Member of the genus brucella do not produce indole, liquefy gelatin, lyses blood, produce acetyl-methyl carbinol or utilize citrate. The methyl red reaction is negative and litmus milk is either unchanged or rendered alkaline (Sci. Committ. An.Hith.An.Welf. 2001’ Raga, 2000).

1.2.4 **Antigenic characteristics**

All smooth brucella strains show complete cross-reaction with each other in agglutination test with unabsorbed polyclonal antisera, across-reaction which does not extent to non-smooth variants. Cross reactions between non-smooth strains can be demonstrated by agglutination tests with unabsorbed anti-R sera. Lipopolysacchride (LPS) comprise the major surface antigens of the corresponding colonial phase involved in agglutination. The S-LPS molecules carry the A and M antigens, which have different quantitative distribution among the smooth brucella strains. This is of value in differentiating biovars of the major species using absorbed monospecific A and M antisera. Serological cross-reactions have been reported between smooth brucella and various other gram-negative bacteria, e.g. *Escherichia coli* 0:116 and 0:157, *Salmonella* group N (0:30) of Kaufmann-White, *Pseudomonas multophilia*, *Vibrio choleras* and especially *Yersinia enterocolitica* 0:9. These organisms can induce significant
levels of antibodies which cross-react with S-LPS brucella antigens in diagnostic tests (Scientific Committee on Animal Health and Animal Welfare, July 2001).

1.2.5 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 phages is a useful test to confirm the identity of brucella spp. And for speciation within the genus. The brucella phages currently used for brucella typing are: Tbilisi (Tb), Weybridge (Wb), and Izatnagar1 (lz1) 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* (see table 2).

1.2.6 Susceptibility to dyes and antibiotics

Susceptibility to the dyes, thionin and basic fuchsin (20ug/ml), which varies between biovars, is one of the routine typing tests of brucella.

On primary isolation, brucellae are usually susceptible in vitro to gentamicin, tetracyclines and rifampicine. Most strains are also susceptible to the following antibiotics: ampicillin, chloramphenicol, cotrimoxazole, erythromycin, kanamycin, novobiocin, spectinomycin and streptomycin, but variation in susceptibility may occur between species, biovars and stains. Most strains are resistant to B-lactamins, cephalosporin, polymyxin, nalidixic acid, amphotericin B, bacitracin, cycloheximide, clindamycin, lincomycin, nystatin and vancomycin at
therapeutic concentration. Penicillin is used for the routine differentiation of the vaccinal strain \textit{Br.abortus} species biovar 1 strain 19, and streptomycin for \textit{Br.melitensis} biovar 1 strain Rev.1, the vaccines widely used for immunization of cattle and small ruminants respectively, from the virulent field strains of their respective biovars by virtue of their different sensitivity to these antibiotics (Alton, Jones, Angus and Verger, 1988).

1.3 Taxonomy of the genus \textit{brucella}

Considering their high degree of DNA homology (>90% for all species), \textit{brucella} have been proposed as a monspecific genus in which all types should be regarded as biovars of \textit{Br. Melitensis} (Verger, Grimont, Grimont and Grayon, 1985). Since proposal has not yet met with complete agreement, the old classification of the genus (and relevant nomenclature) into six species, i.e. \textit{Br.melitensis}, \textit{Br.abortus}, \textit{Br.suis}, \textit{Br.neotomae}, \textit{Br.ovis} and \textit{Br.canis} (Corbel and Brinley-Morgan, 1984), is the classical used world-wide. The first four species are normally observed in the smooth form, whereas \textit{Br.ovis} and \textit{Br.canis} have only been encountered in the rough form. Seven biovars are recognized for \textit{Br.abortus} (1-6 and 9), and five for \textit{Br.suis} (1-5). However \textit{Br.abortus} biovar 8 no longer exist (Meyer and Morgan, 1973), and \textit{Br.abortus} biovar 7 was reported to be mixed culture of \textit{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 \textit{et al.}, 1988 and Corbel, 1990) (see table 3 and 4). Species identification is routinely based on lyses by
phages and on some simple (oxidase, urease...). For *Br. melitensis*, *Br. abortus* and *Br. suis*, the identification at the biovars level is currently performed by four main tests; carbon dioxide requirement, production of H₂S, dye (thionin and basic fuchsin) sensitivity, and agglutination with monospicific A and M antisera. Table (5) show biovars differentiation of brucella spp. Involved in sheep and goats brucellosis. Corbel (1990) mentioned that, there was fairly close relationship between the oxidative metabolism and phage lysis patterns and that both procedures were used for identification of the nomen species. Moreover, a recently developed co-agglutination test, using latex beads coated with a pair of monoclonal antibodies directed against the rough lipo-polysaccharide (R-LPS) and the 25kDa outer membrane protein (omp25), respectively (Bowden, Veger, Grayon and Cloeckaert, 1997), makes it possible to accurately differentiate *Br. ovis* from *Br. canis* and the occasional rough isolates of the smooth brucella species. Intermediate strains are occasionally found due to instability reported for some of the phenotypic characteristics used for current classification of brucella. This situation sometimes impedes the identification of the species and their biovars. Therefore, the identification of the stable DNA-specific marker is considered a high priority for taxonomic, diagnostic and epidemiological purposes. Several methods, mainly PCR-RFLP and Southern blot analysis of various genes or loci, have been employed to find DNA poly-morphism which would enable the molecular identification and typing of the brucella species and their biovars. Among these methods, detection of polymorphism by PCR-RFLP is considered to
have an advantage over Southern blotting, since it is easier to perform and is less
time-consuming when applied to large number of samples. Of all the DNA
sequences investigated by PCR-restriction, the major outer membrane protein
(omp) genes of brucella are the most interesting as they exhibit sufficient
polymorphism to allow differentiation between brucella species and some of their
biovars. More highly conserved brucella genes may also be useful for taxonomic
and epidemiological purpose, even if they contain less polymorphism than the
OMP genes. (See table 5).

Taxonomic knowledge of brucella has progressed a great deal since the techniques
of molecular biology have been applied to these bacteria. A number of molecular
tools (nucleic acid probes, primers..) are now available which make the
elaboration of a more objective and reliable classification of the genus possible.
Judging by the emergence of new brucella types from marine mammals, the genus
is far from being completely identified, (Scientific Committee on Animal Health

Table 1: Differential characteristics of brucella compared to some other
gram-negative bacteria (Alton et al 1988)

<table>
<thead>
<tr>
<th>Test</th>
<th>Brucella</th>
<th>Bordetella</th>
<th>Campylobacter</th>
<th>Moraxella</th>
<th>Acinetobacter</th>
<th>Yersina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bronchiseptica</td>
<td>fetus</td>
<td>Diplococcoid</td>
<td>Diplococcoid</td>
<td>Rod</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------</td>
<td>-------</td>
<td>--------------</td>
<td>--------------</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td>Small coccobi</td>
<td>cilli</td>
<td>Small coccobi</td>
<td>Comma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small coccobi</td>
<td>cilli</td>
<td>Comma</td>
<td>Diplococcoid</td>
<td>Diplococcoid</td>
<td>Rod</td>
</tr>
<tr>
<td>Motility at 37c</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Motility at 20c</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lactose fermentation on macConkey agar</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Va</td>
<td>V</td>
<td>-</td>
</tr>
<tr>
<td>Acid production on agar containing lactose</td>
<td>-b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>Haemolysis on blood agar</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>-</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+c</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>+d</td>
<td>+</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+c</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>-</td>
</tr>
<tr>
<td>Agglutination with: S-brucella antiserum</td>
<td>+f</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>R-brucella antiserum</td>
<td>+g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2: Differential characteristics of brucella phages

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>LYSIS BY PHAGES (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tb</td>
</tr>
<tr>
<td>Br. Melitensis</td>
<td>-</td>
</tr>
<tr>
<td>Br. ovis</td>
<td>-</td>
</tr>
<tr>
<td>Br. abortus</td>
<td>+</td>
</tr>
</tbody>
</table>

(1) at the routine test dilution

Table 3: biovar differentiation of the species of the brucella according to Alton et al 1988
<table>
<thead>
<tr>
<th>Species</th>
<th>Biovar</th>
<th>Co2 requirement</th>
<th>H2S production</th>
<th>Growth on dyes a thionin</th>
<th>Basic fuchsin</th>
<th>Agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br.melitensis</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Br.abortus</td>
<td>1</td>
<td>+c</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+c</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+c</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+c</td>
<td>+</td>
<td>-</td>
<td>+d</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Br.suis</td>
<td>1</td>
<td>t or-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-e</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-f</td>
<td>+</td>
</tr>
<tr>
<td>Br.neotomae</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Br.ovis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-f</td>
<td>-</td>
</tr>
<tr>
<td>Br.canis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-f</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Biovar</th>
<th>Co2 requirement</th>
<th>H2S production</th>
<th>Growth on dyes a thionin</th>
<th>Basic fuchsin</th>
<th>Agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br.melitensis</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Br.abortus</td>
<td>1</td>
<td>+c</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+c</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+c</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+c</td>
<td>+</td>
<td>-</td>
<td>+d</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Br.suis</td>
<td>1</td>
<td>t or-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-e</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-f</td>
<td>+</td>
</tr>
<tr>
<td>Br.neotomae</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Br.ovis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-f</td>
<td>-</td>
</tr>
<tr>
<td>Br.canis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-f</td>
<td>-</td>
</tr>
</tbody>
</table>

a = dye concentration, 20μg/ml in serum dextrose medium (1:50000).
A = A mono-specific antiserum; M = M mono-specific antiserum; R = rough brucella antiserum.
C = usually positive on primary isolation
d = some strain do not grow on dyes
e = negative for most strain
f = negative for most strain
g = growth at 10ug/ml (1:100000) thionin

Table 4: classification of the genus brucella according to corbel 1990:
<table>
<thead>
<tr>
<th>designation</th>
<th>thionin</th>
<th>Basic fuhsin 20ug/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br.melitensis biovar melitensis</td>
<td>1 2 3</td>
<td>- - -</td>
</tr>
<tr>
<td>Br.melitensis biovar abortus</td>
<td>1 2 3 4 5 6 7</td>
<td>(+) (+) (+) (+) - -</td>
</tr>
<tr>
<td>Br.melitensis biovar suis</td>
<td>1 2 3 4 5</td>
<td>- - -</td>
</tr>
<tr>
<td>Br.melitensis biovar ovis</td>
<td>Br.ovis</td>
<td>+ -</td>
</tr>
<tr>
<td>Br.melitensis biovar canis</td>
<td>Br.canis</td>
<td>- -</td>
</tr>
<tr>
<td>Br.melitensis biovar neotomae</td>
<td>Br.neotomae</td>
<td>- +</td>
</tr>
</tbody>
</table>

*more differentiation of brucella abortus biovar 3 and **some strain are inhibited basic fuchsin.
***some isolate are resistant to basic funchsin
(+) most strain positive.
(-) most strain negative
1.4 Brucellosis

1.4.1 Definition

Brucellosis is a contagious disease of animal, which is transmitted to man (anthropozoonosis), (Carpenter and Hobbert, 1963).

1.4.2 Aetiology
Cattle

Cattle are the primary host of *brucella abortus*. *Br.melitensis* can infect cattle where abortion is very rare (Nicoletti, 1980). It become carrier and excretes the organism in milk. Cattle is resistant to generalized infection with *Br.suis*, but they sometimes acquired inapparent infections which causes them to react to routine tests in eradication campaign (Alton, 1990), it occasionally found localized in bovine udder, excreted in milk, and this could cause infection to man (Brich and Gliman, 1934).

Camels

Camels are susceptible to both *Br.abortus* and *Br.melitensis* (Nicoletti, 1989). Rutter and Mark (1963) reported that camels could be infected by any of the three classical species of brucella, but in Africa, *Br.melitensis* was the predominantly isolated organism, (Tag ElSir, 2002).

Sheep and goats

Sheep and goats are the primary host of Br.melitensis. it was the first species of the genus brucella, described. It was first isolated by Bruce 1887 (Alton, 1990). Infection of sheep and goats with with *Br.ovis* frequently causing epididymitis and infertility in rams and occasionally abortion in ewes (Mel Hopkirk, 1995). (See table 5).

1.4.3 Transmission of the disease

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

1.4.3.2 Mode of infection

According to Buxton and Fraser (1977) the disease is transmitted from infected animal or contaminated material to susceptible one through mucous membrane of the alimentary and respiratory tract, the conjunctiva and broken skin, artificial insemination and through the vagina in some species. The dogs have been shown to be mechanical and biological vectors of brucellosis (Jiont FAO/WHO Expert Committee on Brucellosis, 1986). Insect also act as vector of infections (Corbel, 1989). In ovine brucellosis, spread of infection is mainly venereal or direct from ram to ram (brucellosis can established in the uterus of pregnant ewes, however is only carried for the term of that pregnancy), (Nancy, 2002).

1.4.3.3 Survival of brucella in the environment

Compared with most 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 (table 6). Thus when pH> 4, high humidity, low temperature and absence of direct sunlight, brucella may retain infectivity for several months in water, aborted foeti and fetal membranes, faeces and liquid manure, wool, hay, on building, equipment and clothes. Brucellae are able to withstand drying particularly in the presence of extraneous organic material and will remain viable in dust and soil. Survival is prolonged at low temperature, especially below 0°C (Alton, 1985; Joint FAO/WHO Committee, 1986; Nicoletti, 1980). The organisms are susceptible to an acid pH, disinfectants and direct sunlight.

Survival of brucella in milk and diary products is related to a variety of factors including the type and age of product, humidity level, and temperature, change in pH, moisture content, biological action of other bacteria present and condition of storage. The result of some studies (Carrere, Lafenetre, Quatrefages, Noronha, 1960; Plommet, Fensterbank, Vassal, Auclair, Mocquot, 1988), are present in table (7). Brucella does not persist for along time in ripened fermented cheese. The optimal fermentation time to ensure safety is not known, but is estimated at 3 months (Nicoletti, 1989). However, in normally acidified soft cheese, the strictly lactic acid and short-time fermentation and drying increase the survival time of brucella. Additionally, the process of manufacturing ice-cream not destroy brucella. Previous pasteurization of milk or cream is the only means to ensure safety of these products. In contrast to diary product, the survival time of brucella in meat seems extremely short, except in frozen carcases where the
organisms can survive for years. The number of organisms per gram of muscle is small and rapidly decreases with pH drop of the meat. *Brucella suis* has been isolated from hog carcases after 21 days of refrigeration, (Manson-Bahar and Apted, 1982).


<table>
<thead>
<tr>
<th>ENVIRONMENT</th>
<th>CONDITION</th>
<th>SURVIVAL TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sunlight</td>
<td>&lt;31°C</td>
<td>4h30</td>
</tr>
<tr>
<td>Water</td>
<td>-4°C</td>
<td>4 months</td>
</tr>
<tr>
<td>Water (laboratory)</td>
<td>20°C</td>
<td>2.5 months</td>
</tr>
<tr>
<td>Water (lake)</td>
<td>37°C, pH=7.2</td>
<td>&lt;24 hours</td>
</tr>
<tr>
<td></td>
<td>08°C, pH=6.5</td>
<td>&gt;2 months</td>
</tr>
<tr>
<td>Soil</td>
<td>Dried in laboratory</td>
<td>&lt;4 days</td>
</tr>
<tr>
<td></td>
<td>Dried at 18°C</td>
<td>69-72 days</td>
</tr>
<tr>
<td></td>
<td>Wet</td>
<td>&lt;7 days</td>
</tr>
<tr>
<td>Urine</td>
<td>Humid atmosphere</td>
<td>&gt;2 months</td>
</tr>
<tr>
<td></td>
<td>Autumn (90% humidity)</td>
<td>48-73 days</td>
</tr>
<tr>
<td>Raw milk</td>
<td>February (rapid drying)</td>
<td>72 days</td>
</tr>
<tr>
<td></td>
<td>37°C, pH=8.5</td>
<td>16 hours</td>
</tr>
<tr>
<td>Whey</td>
<td>08°C</td>
<td>6 days</td>
</tr>
<tr>
<td></td>
<td>-40°C</td>
<td>24 hours</td>
</tr>
<tr>
<td></td>
<td>17-24°C</td>
<td>48 hours</td>
</tr>
<tr>
<td>Product</td>
<td>Species of Brucella</td>
<td>Temperature</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Milk</td>
<td>Br. abortus</td>
<td>71.7</td>
</tr>
<tr>
<td></td>
<td>Br. abortus</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Br. abortus</td>
<td>25-37</td>
</tr>
<tr>
<td></td>
<td>Br. abortus</td>
<td>0</td>
</tr>
<tr>
<td>Cream</td>
<td>Br. abortus</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Br. melitensis</td>
<td>4</td>
</tr>
<tr>
<td>Ice cream</td>
<td>Br. abortus</td>
<td>0</td>
</tr>
<tr>
<td>Butter</td>
<td>Br. abortus</td>
<td>8</td>
</tr>
<tr>
<td>Cheese</td>
<td>various</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 7: studies on brucella survival time in diary products (Carrere et al 1960; Daveies and Casey, 1973; Nicoletti, 1989; Plommet et al., 1988)
<table>
<thead>
<tr>
<th>various</th>
<th>Br.melitensis</th>
<th>-</th>
<th>-</th>
<th>15-100 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>feta</td>
<td>Br.melitensis</td>
<td>-</td>
<td>-</td>
<td>4-16 days</td>
</tr>
<tr>
<td>pecorino</td>
<td>Br.melitensis</td>
<td>-</td>
<td>-</td>
<td>&lt;90 days</td>
</tr>
<tr>
<td>Roquefort</td>
<td>Br.abortus and Br.melitensis</td>
<td>-</td>
<td>-</td>
<td>20-60 days</td>
</tr>
<tr>
<td>Camembert</td>
<td>Br.abortus</td>
<td>-</td>
<td>-</td>
<td>&lt;21 days</td>
</tr>
<tr>
<td>Erythream</td>
<td>Br.melitensis</td>
<td>-</td>
<td>-</td>
<td>44 days</td>
</tr>
<tr>
<td>Cheddar</td>
<td>Br.bortus</td>
<td>-</td>
<td>-</td>
<td>6 months</td>
</tr>
<tr>
<td>White</td>
<td>Br.melitensis</td>
<td>-</td>
<td>-</td>
<td>1-8 weeks</td>
</tr>
<tr>
<td>whey</td>
<td>Br.abortus</td>
<td>17-24</td>
<td>4.3-5.9</td>
<td>&lt; 4 days</td>
</tr>
<tr>
<td></td>
<td>Br.bortus</td>
<td>5</td>
<td>5.4-5.9</td>
<td>&gt;6 days</td>
</tr>
</tbody>
</table>

1.4.4 Pathogenesis and immune response

Pathogenically, *Br.melitensis* infection is sheep and goats is similar to *Br.abortus* in cattle, differences are significant, and each species of brucella causes a different disease (OIE Manual, 1996). The virulence of brucella varies between species and strains.

Brucellae are facultative intracellular parasites of the reticulo-endothelial system. Infection occurs mainly through the mucous membranes of the oropharynx, upper respiratory tract and conjunctiva. Other potential routes of infection are through the mucous membranes of the male and female genial tract. After gaining entrance to the body the organism succeed in arriving via the lymph
channel at the nearest lymph node. When the bacteria prevail over the body defense, a bacterimia is generally established. In pregnant animals the uterus is invaded resulting in abortion. The udder is an important predilection site for brucella. The infection also becomes established in various lymph nodes and organs (Scientific Committee on Animal Health and Animal Welfare, July 2001). The major clinical sign is abortion, hygroma, orchitis, arthritis, epididymitis, metritis, retention of placenta, weak or still births (Blood and Radostits, 1989; Musa, Jahans and Fadalla, 1990), while in camels the disease generally is not accompanied by clear-cut symptoms (Mustafa, 1987), and occur without obvious signs. Generally infection do not persist more than four years (Solonitsuin, 1949).

In man the disease is caused by direct or indirect contact wit infected animals and the infection usually causes severe or chronic illness (Raga, 2000).

Humoral as well as cell mediated immune mechanisms are activated in infected animal. The serological response is transient and sometimes missing in young sexually immature animals.

1.4.5. Diagnosis

Many workers used serological tests for diagnosis of the disease. A definitive diagnosis of brucellosis requires the isolation and identification of the etiological agent (Davis, Dulbecco, Eisen and Ginsberg, 1980; Volk, 1982). Several methods are used for diagnosis of brucellosis and include:

1.4.5.1 Direct smear
Smear made direct from product of abortion or calving or vaginal swabs can be stained by Koster or modified Ziel-Neeelsen method. Neither staining technique is specific for brucella species and will produce positive result with Coxiella burnetti. The brucella organism stains pink or red against a blue background (Corbel, 1973).

1.4.5.2 Culture of samples to isolate the causative agent

It is the accurate methods for the diagnosis of brucellosis in animal and man. The most suitable samples for isolation of the organism are fetal membranes, fetal stomach content, milk, vaginal swabs and ram semen. The spleen and lymph nodes (iliac, mammary and prefemoral) are the most reliable samples for isolation purpose in necropsied animals. Several media are suitable for the isolation of brucella, such as: Serum dextrose agar (SDA), Serum tryptose agar, Brucella agar. Farrell's selective medium, developed for the isolation of *Br. abortus* from milk (Farrell, 1974), also recommended for isolation of *Br. melitensis*.

*Br. Abortus* is the only strain that requires supplementary (5%-10%) carbon dioxide for growth especially on primary isolation (Cruickshank, Duguid, Marmion and Swain, 1982).

1.4.5.3 Guinea pig inoculation

More successful than direct culture especially contaminated material and small number of organisms. Guinea pigs are injected intramuscular or intraperitoneal if the emulsified material is free from contamination, and killed after 4-5 weeks of inoculation. Typical lesions include necrotic foci in liver, spleen,
lymph nodes and orchitis in male guinea pigs. The spleens are cultured on SDA and the sera are subjected to SAT. Recovery of the organism from the spleen or positive serum agglutination test is a justifying diagnosis of brucellosis (Alton, Lois, M.Jonas and Pietz, 1975).

1.4.5.4 Other method for agent detection

While culturing is a specific method, its sensitivity depends on the viability and numbers of brucella within the sample, the nature of samples (fetal organ, fetal membranes, lymph nodes, etc) and the number of specimens tested from the same animal (Hornitzky and Searon, 1986). The time required for culturing field specimens can be long and tissues or fluids that are only contaminated with low number of brucella may not be detected. Thus in the case of tissues or fluid contaminated with non viable or a low number of brucella, PCR could be a potentially useful method for the diagnoses of brucellosis. Several authors reported a good sensitivity of PCR for detection of brucella DNA on pure culture (Fekete, Bantle, Halling and Sanborn, 1990a, 1990b; Baily, Krahn, Drasar and Stocker, 1992; Herman and De Ridder, 1992; Romero, Pardo, Lopez-Goni, 1995a; DaCosta, Guillou, Garin-Bastuji, Thiebaud, Dubray, 1996).

1.4.5.5 Serological tests

Several serological techniques, with variable degree of sensitivity and specificity, have been used to detect the presence of antibodies to brucella in animals and humans and are presented as follow:

1.4.5.5.1 Rose Bengal Plate Test (RBPT)
The RBPT is a commonly used screening test for brucella agglutinins in animals and humans (Brinley-Morgan, Mackinnon, Gill, Gower and Norris, 1981). It is a modification of the acid-plate agglutinin test, employing a suspension of Br. abortus organism stained with Rose Bengal dye and buffered at pH 3.65 (Corbel, 1972). Sera showing positive reactions are then subjected to further tests such as the serum agglutination test (SAT) or complement fixation test (CFT) to provide quantitative titres. The results obtain by the (RBPT) show excellent agreement with the CFT. Diaz, Maravi, Delgado and Rivero, (1978), in their study using RBPT and counter-immunoelectrophoresis (CIE) testing the diagnosis of brucella meningitis found that the RBPT was positive in five of five patients studies and CIE was positive in four of five patients studied. In study comparing a variety of tests of used in the serological diagnosis of human brucellosis RBPT and CIE were demonstrated to be better than other tests such as the slide method and immunodiffusion test (Mandell et al., 1979). Diaz Aparicio et al. (1994) employed five tests for the diagnosis of brucellosis in goats; the test include RBPT, CFT, Enzyme-Linked immunosorbent Assay (ELISA), Radial Immunodiffusion (RID) and Counter-Immunoelectrophoresis (CIEP). They found that the sensitivity was 100% for RBPT, 94% for CFT and ELISA and 93 % for RID. All tests were 100% specific because they gave negative results when testing sera from brucella-free goats. The RBPT identifies infected animals or humans earlier than do other serological tests especially the SAT. The antibodies active in the RBPT are immunoglobulin of the IgG1 classes (Corbel, 1972). However,
where the incidence of the disease is low and calf vaccination with strain 19 is widely used, the test is too sensitive and positive results must be confirmed by standard tube agglutination and CFT (Brinley-Morgan et al., 1981).

1.4.5.5.2 Serum Agglutination Test (SAT)

The test was first introduced by Wright and Smith in 1897 and is the most widely used diagnostic test for brucellosis (Chappel, McNaught, Bourki and Allan, 1978; Siddiqui, 1985). Alton and Jones (1967) standardized the techniques of the serum agglutination test together with the preparation of the antigens. The SAT allows for the determination of the serum antibody titre. The test detects both IgM and IgG antibodies. In chronic infections the test may be inconclusive or negative (Brinley-Morgan et al., 1981). The test is also less sensitive than the RBPT in detecting early infection in animals or man because of low antibody levels (Brinley-Morgan et al., 1981).

1.4.5.5.3 Complement Fixation Test (CFT)

The CF test is the most widely used test for serological confirmation of brucellosis in animals. It has been shown to be an accurate and sensitive diagnostic test. Serum agglutination antibody tends to decrease in chronic infection, however, the complement fixing antibodies persist at diagnostic levels. The complement fixation test is used in differentiating antibodies due to an active chronic infection or antibodies due to recent vaccination (usually negative).
The CFT has many drawbacks such as complexity, variability of reagents, prozones, anticomplementry activity of sera, difficulty to perform with hamolysed sera and subjectivity of the interpretation of low titers. Therefore, while the sensitivity of RB is sufficient for the surveillance of free areas at the flock level, RB and CF should be used together in infected flocks to obtain accurate individual sensitivity in test-and-slaughter programmes moreover, an important drawback of both RB and CF tests is their low specificity when testing sera from sheep and goats vaccinated subcutaneously with Rev.1 (Fensterbank, Pardon and Marly, 1982; Jimenez de Bagues, Marin, Blasco, Moriyon and Gamazo, 1992; Diaz-Aparicio et al., 1994).

1.4.5.5.4 Enzyme-Linked Immunosorbent Assay (ELISA)

It is known under a variety of names such as enzyme immunoassay (EIA) (Van-Weem and Schuurs, 1971), enzyme labeled assay (ELA) (Saunders and Wilder, 1974,) competitive enzyme linked immunoassay (CELIA) (Yarde, Sasse, Wang, Hussa and Garancis, 1976) and enzyme-linked immunosorbent assay (ELISA) (Engvall and perlmann, 1971).

The ELISA is perhaps the most useful term since it identifies the heterogenous enzyme assay and at the same time clearly differentiate it from tests which employ antibody peroxidase conjugates for staining reaction for microscopy (Voller, Bartlett and Bidwell, 1978).

Carlsson, Lindberg and Hammarstrom, (1972) were the first to realize that ELISA technique could be used in the field of diagnostic bacteriology, when they
detected, quantitatively, anti-salmonella O-antibodies in human sera. Holmgren and Sevennerholm (1973) applied the procedure to the assay of Vibrio cholerae. Since it is development the ELISA technique has proved to be highly sensitive and specific and capable of being applied to immunodiagnose numerous infectious diseases. Several studies have dealt with the application of ELISA to the immunodiagnoses of brucellosis, and the data from these studies have allowed the investigators to propose it as a method of choice for the serological diagnosis of brucellosis (Voller et al., 1978). The ELISA can distinguish between IgG and IgM (Magee, 1980). Sutherland, Evan and Bathgate, (1986) compared ELISA with CFT and RBPT for detecting antibodies to Br. abortus. Their results showed that ELISA was more sensitive than CFT and RBPT particularly in herds where Br. abortus biotype 2 was present. They recommended that ELISA should be used together with CFT in eradication programmes.

There are two main steps on which ELISA is dependent. Firstly, the soluble antigen or antibody is rendered insoluble by adsorption to a solid phase such as polystyrene tubes or microtitre plates. Secondly, the antigen or antibody is conjugated to an enzyme without loss of activity. The ELISA can be read either visually or spectrophotometerically (Baker, 1980).

**1.4.5.5. Antiglobulin (Coombs') Test**

The antiglobulin test or Coombs' test (Coombs',Mourant, Race, 1945) was developed to detect antibodies which, although they combine with cellular antigens of brucella, do not give rise to agglutination. The presence of these so
called "incomplete agglutinins" can be detected by using antibody directed against the lgG fraction of the animal species being tested (MacMillan, 1990). In 1955, the test was described as the most effective immunological method for detection of brucellosis in goats (Esteban, 1959). The test is useful in chronic cases containing non-agglutinating antibodies (Elberg, 1983). It is not recommended in bovine vaccinated with strain S19 (MacMillan, 1990), or in small ruminants immunized with Rev. 1 vaccine (Farina, 1985), because of its low specificity as compared to the CFT.

1.4.5.6 Test for Detecting Antibodies in Milk

1.4.5.6.1 Milk Ring Test (MRT)

The MRT is widely used as a herd test to detect the presence of brucellosis. It was first introduced by Fleishauer (1937) in Germany as screening test. The MRT depends on the presence of a fat globule agglutinin present in the milk which upon reaction with the brucella antigen will aggregate and rise to the top. These globule agglutinins are detected by adding to the milk a brucella antigen stained with haematoxyline (blue colour) or tetrazolium (red colour), (Alton et al., 1975 and Brinley-Morgan et al., 1981). These two stains produce similar results (Alton et al., 1975). The brucella antigen combines with the brucella agglutinin, to form a complex that adheres to the fat globules and rise with them to form a blue coloured cream layer. If there are no antibodies present, the stained brucella antigens remain in suspension in the milk column below the white cream layer (Brinley-Morgan et al., 1981). With sheep and goats milk the agglutinated antigen
usually falls to the bottom of the tube, but occasionally it may rise with fat globules and produce a ring as occur with cow milk (Alton et al., 1975).

1.4.5.6.2 Whey Agglutination Test (WAT)

Whey agglutination test was first introduced by Hall and Learmonth (1933). It is usually employed as a confirmatory test because it is rarely affected by vaccination (Cruichshank, Duguid, Marmion and Swain, 1982). It is also less influenced than MRT by non-specific factors such as excessive heating, as temperature above 45°C for over 5 minutes will lead to a decrease in brucella antibody content (Brinley-Morgan et al., 1981).

After [reparation, whey is then subjected to a standard tube agglutination test as for serum (TAT). Any reaction of more than or equal to 20 IU is usually considered as indicative of infection (Brinley-Morgan et al., 1981).

1.4.5.7. Allergic Skin Test (AST)

It is routinely and officially used for the diagnosis of brucellosis in East European countries, Greece, Cyprus the former USSR, china, Mongolia, Germany, Italy and France (Kolar, 1990). Kolar mentioned that the test could be used in farm animals, but it was mainly intended for sheep, goats and pigs. In cattle the test could be used to confirm or correct the result of the serological tests. In some countries, it was found to be equivalent to serological test in cattle (Jerabek, 1962). Kolar (1990) stated that (AST) was proved useful in camels and horses in Mongolia and the former USSR.
(AST) is performed strictly into the skin. The site of injection depends on the animal species. The test is specific and does not react to cross reacting organisms (Kolar, 1990). Some workers believe that AST more sensitive than the serological tests (Kolar and Kolarova, 1955). The drawback of the test is that the hypersensitivity infected animals may persist for several years or lifetime. This complicates the interpretation of the test.

1.4.5.8 Control and Eradication of Brucellosis

There is a justification to control or eradicate brucellosis, because it is a serious zoonosis, and it is difficult to be cured because the organism has the capacity to grow intracellular. Plu mmet (1986) recommended three ways for control and prevention of brucellosis.

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 seropositive animals.

Nicoletti (1980) stated that maximum control and prevention achieved when the three ways above are combined.

The Joint Food and Agriculture Organization and the World Health Organization Expert Committee on brucellosis (1986) recommended the following strategies for the control of brucellosis in cattle, sheep, goats, swine and dogs:

**Control in cattle**
A- Mass immunization using the strain 19 vaccine, at the age four months, is the only way to reduce brucellosis in high prevalence areas. Montefiore, Alausa and Tomori, (1984) reported that the vaccination of cattle with strain 19 affords protection for up to 7 years.

B- Eradication by test and slaughter:

1. All cattle, female and bulls over one year old, are tested for brucella antibodies using RBPT and SAT or CFT. The positive reactors are to be removed and the herd is to be retested after 30-60 days. If no reactors are found on the first retest then the herd is subjected to a second retest six months after the first retest. The herd is declared brucellosis-free if there are no reactors on the second test.

2. Reactors should be removed from the herd as soon as possible and slaughtered.

3. The sale of female cattle over one year old from infected herds should be prohibited unless they are to be send for direct slaughter.

4. Regulations to prevent the introduction of infection to brucellosis-free premises are also required.

In areas where herds are large, it may be difficult to apply the regimen of test and slaughter. Instead the heifer segregation plan is applied. In this plan
heifers are tested at weaning and reactors are slaughtered. The negative heifers are
immunized. Six weeks later they are again tested and any further reactors are
removed.

Control in sheep and goats

Eradication by test and slaughter is feasible where *brucella melitensis* is
introduced into a previously non-infected region or where the prevalence is low.
However, where *Br. melitensis* is endemic control by Rev.1 vaccine is
recommended. The measures outlined for eradication of brucellosis in cattle can
be applied to the readication of *Br. melitensis* infection in sheep and goats, with the
following exceptions:

1. Infected and non-infected flocks can be identified by the use of brucellin
   skin test. Positive brucellin reactions should be investigated for
   antibodies using the serological tests aforementioned.

2. In flocks immunized with Rev.1 vaccine, the animals can be tested one
   years or more after immunization provided that the CFT is used.

3. Where communal grazing is practiced all animals should be tested.

4. Surveillance in brucellosis-free areas can be achieved by periodic
   serologic testing on all sheep and goats in the area.

1.4.5.9 Treatment of Brucellosis

The use of long acting oxytetracycline at 20 mg/kg body weight
intramuscularly at 3-4 days intervals for 5 treatments in combination with
streptomycin at 25 mg/kg body weight intramuscularly or intravenously daily for
seven consecutive days was partially successful in the treatment of infected cows. Radwan, Hafez, Al-Aska, Yamani, Bekairi, Al-Julaifi and Al-Mukayel (1987) pointed out that a long term treatment with a high dose of oxytetracycline (1000 mg/day/six week, I/P) had completely eliminated \textit{Br.melitensis} from naturally infected sheep. In humans however, many antimicrobial agents are used such as \textit{tetracycline} or \textit{doxycycline}, \textit{trimethoprim}, \textit{sulfamethoxazole} and \textit{streptomycine} (Young, 1989).

\textbf{1.4.5.10 Vaccination}

Vaccination is often the first step in the control of infectious diseases.

\textbf{1.4.5.10.1 \textit{Br.abortus} S 19 vaccine}

\textit{Br.abortus} S19 vaccine was found by Buck (1930). The common age for S19 vaccination is 2-10 months depending upon the breed of cattle. Such a procedure was adopted to avoid persistent agglutinins. Calves between 3-6 months are usually vaccinated but, in some cases, the upper limit can be extended to 8 months (WHO Expert Committee on Brucellosis, 1986). Lambert, Amerulf, Manthei and Goode (1961) and King and Frank (1961) showed that there was no difference in the degree of protection when S19 was given at three, four, six, eight or nine months of age. Redman, Deyoe and King (1967) conclude that the older the animal at the time of vaccination the longer the agglutination titre tend to persist. In the usual S19 vaccination, at 4-6 months, the post vaccinal response lasts 6-12 months. As a result immunization at that age does not affect serological tests on adults (Fensterbank and Plommet, 1979). S19 vaccine is usually
administered subcutaneous (Buck, 1930) or conjunctively (Plommet and Fensterbank, 1976). The same investigators notice that a booster dose administered conjunctively 6 months after the standard S19 subcutaneous injection improved the immunity. The usual dose of young calf 3-8 months was calculated to be 50-120×10^9 viable bacteria. Recently, it was found that a dose of 3-10×10^9 viable bacteria in 2 ml produce similar protection (WHO Expert Committee on Brucellosis, 1986). Adult cattle can be vaccinated with a reduce dose of S19 vaccine i.e. 3× 10^8 CFU, without reducing the immunity. The protection rate of S19 vaccine was said to be 65-70% (Nicoletti, 1980).

1.4.5.10.2 **Br.abortus 45/20 vaccine**

In Ireland, rough *Br.abortus* killed with an adjuvant 45/20 is used for cattle nine months or above, irrespective of their pregnancy status. This vaccine has few diagnostic problems, safe and stable.

1.4.5.10.3 **Br.melitensis Rev.1 vaccine**

The Rev.1 vaccine is presently recognized as the best available vaccine for the prophylaxis of brucellosis in sheep and goats. The duration of immunity conferred by vaccination with Rev.1 could be considered lifelong (Alton, 1966, 1968). The Rev.1 vaccine may be administered by the standard method (1-2× 10^9 CFU s S/C) or may be administered by the conjunctival route (0.5-2× 10^9 CFU). A disadvantage of Rev.1 is long lasting serological response which causes obvious problem for the application of combined vaccination and test and slaughter
The vaccination of pregnant animals with standard Rev.1 doses s/c is followed by vaccine induces abortion in many animals (Alton and Elberg, 1967; Elberg, 1981; Blasco, 1997). Localization in the udder and shedding in milk also occurs (Hagan and Bruner, 1988). A reduction of the vaccine dose induces a shorter and less intense antibody response following vaccination (Gasca, Jimenez and Diaz, 1985). Accordingly, a reduced dose ($10^3$-$10^6$ CFU) has been used subcutaneously in field trials and reported as an effective method to control brucellosis in small ruminants and being relatively safe in pregnant sheep and goats (Kolar, 1984; Gasca et al., 1985; Alkhalaf, Mohamed and Nicoletti, 1992). This method of immunization has demonstrated its efficacy in well-controlled experiments (Gasca et al., 1985). However, field trials and controlled experiments have demonstrated that reduced doses of Rev.1 vaccine may induce vaginal excretion and abortion in pregnant sheep and goats after field infections (Alton, 1970 and Fensterbank et al., 1982). It was noted that the level of protection was poor in goats which received a dose of $10^4$ CFU (Alton, 1970). When vaccinating pregnant sheep and goats the conjunctival method results in fewer abortions compared to the S/C method. However, a significant proportion of sheep and goats vaccinated conjunctivally with standard doses excrete Rev.1 vaccine and abort (Zundel, Verger, Grayon and Michel, 1992).

**1.4.5.10.4 H38 vaccine**

A killed adjuvant vaccine H38 is used for sheep and goats.

**1.4.5.10.5 Br.suis S2**
*Br.suis* strain 2 (S2), a classically obtained brucella attenuated strain with smooth LPS. It was attenuated by serial transfers on media over a period of years (China Control Institute of Veterinary Bioproducts and pharmaceuticals, 1978). Oral vaccination of sheep, goats, cattle and pigs by S2 vaccine was found to be better than either subcutaneous or intramuscular inoculations. There were no serious post vaccinal reactions, abortion of pregnant animals or provocation of strong serological reactions using S2 vaccine given orally (China Control Institute of Veterinary Bioproducts and pharmaceuticals, 1978). However, this vaccine showed no protective effect against *Br.melitensis* in sheep in fully controlled experimental conditions (Verger, Grayon, Zundel, Lechopier, Olivier-Bernardin, 1995).

1.4.5.10.6 *Br.abortus* RB51

Live attenuated but rough brucella strain (Schurig, Roop, Bagchi,Boyle, Buhrman, Sriranganathan, 1991). It is equally effective as S19 vaccine in protecting against *Br.abortus* in cattle. Preliminary experiments suggest that this vaccine can be effective for the prophylaxis of *Br.melitensis* infection in goats (Suarez, Soberon, Diaz-Aparicio, Adams, 1998).

1.4.5.11 the Situation of Brucellosis

1.4.5.11.1 The situation of Brucellosis Worldwide

Brucellosis has a worldwide distribution with documented studies from Mexico, Central and South America, much of Africa, Europe, the Middle East, India, China, the Union of Soviet Socialist Republic, Australia, Polynesia and
New Zealand (Elberg, 1981 and 1983; Braude, Davis and Fierer, 1986). In the United States most of the disease is due to *Br. abortus*, but some cases due to *Br. melitensis* have occurred in goats-raising areas and only a few cases due to *Br. suis* have occurred (Smith, 1984). The successful eradication program of bovine brucellosis in the United States has markedly reduced the incidence of human brucellosis from 3500 cases reported in 1950 to 183 cases in 1981 (Braude, Davis and Fierer, 1986). In Britain indigenous disease caused by *Br. melitensis* is unknown. An official survey undertaken in England and Wales in 1960-1961 indicates that 25-30% of all dairy herds were infected (Wilson, 1975). The progressive fall in the incidence of the disease and its eventual elimination. Scotland was declared free from brucellosis on 1 January 1980 followed by England and Wales on 1 November 1981 (Smith, 1984).

*Br. melitensis* infection in sheep appears to occur endemically in the Mediterranean region, especially along its northern and eastern shores, stretching through central Asia as far south as the Arabian Peninsula and as far east as Mongolia. Parts of Latin America are also seriously affected. Brucellosis caused by *Br. melitensis* also occurs in Africa and India (FAO/OIE/WHO, 1997).

1.4.5.11.2 The situation of Brucellosis in the Sudan

Brucellosis in the Sudan was first reported in a diary farm in Khartoum, where *Br. abortus* was isolated from an aborted Friesian cow (Bennet, 1943). After that many investigators isolate the organism from cattle in many parts of the country (Khan, 1956; Daffalla, 1962; Shigdi and Razig, 1971-1973; Ibraim, 1974;
Musa and Mitchell, 1985; Khalafalla, Dafalla and Bakhiet, 1987). Musa (1995), isolate the organism from cattle in Darfur States and found that positive reactors to the serological tests carried out in different areas of Darfur State in all animal species were as follow: Cattle 13.9%, camels 7.8%, sheep 3.5%, goats 5.98%, horses 4.9% and donkeys 3.6%.

The disease was also diagnosed serologically in many other parts of the country, in Fung District (Mustafa and Hassan, 1969), at Wadi Halfa, Northern Province (Abdulla, 1966), in Malakal and Tong, Southern Sudan, in El obied dairy farm, Western Sudan and in Kenana cattle at Singa in the Blue Nile Province (Dafalla and Khan, 1958).

1.4.5.11.3 The situation of bovine brucellosis in the Sudan

Bovine brucellosis was first diagnosed by Bennett (1943), who isolated \textit{Br. abortus} from an abortus from an aborted foetus of a cow in a dairy farm near Khartoum. The report by Bennett (1943) led to extensive serological survey to detect antibodies to brucella in cattle, camels, sheep and goats in most parts of the country. As a result of several cases of undulant fever among European residents in Barakat in ElGezira area in 1953, the cows supplies the milk were examined for antibodies to brucella. Many cows gave positive reaction to SAT and \textit{Br. melitensis} was isolated from the milk of one of them. \textit{Br. melitensis} was also isolated from milk of a sheep and goats sharing grazing with the cattle (Dafalla and Khan, 1958). Bovine brucellosis was serological diagnosed in dairy herds in Malakal and Tong in the Southern Sudan in 1953, in Elobied dairy in Western Sudan and in
Kenana cattle at Singa in the Blue Nile Province (Dafalla and Khan, 1958). The disease was also serologically diagnosed in the Upper Nile Province in Southern Sudan. Abdulla (1966) examined 298 cattle at Wadi Halfa in the Northern Province and reported that 3% of the animals were positive. Mustafa and Hassan (1969) found that in the Fung District of the Blue Nile Province the number of positive cattle in the East and West bank of the Blue Nile was 8.7% and 5.7%, respectively. Brucellosis was also serologically diagnosed in various parts of the country by other workers (Ibrahim and Habiballa, 1975; Suliman, 1987; Musa, 1995; and Raga, 2000).

1.4.5.11.4 The Situation of Camels Brucellosis in the Sudan

Since 1971 brucella infection has been reported in camels by Mustafa and Awad Elkarim followed by Hussien and Saad, (1975) who surveyed the disease in Kordofan Province. Camels' brucellosis was also serologically diagnosed in various parts of the country by several workers. In Kassla (1971), Mustafa and Awad Elkarim tested 171 camels; three were positive and two suspicious for brucellosis. While in Butana eight were positive out of 139 camels tested. Survey in Gash and Tokar showed prevalence of Br. abortus antibodies was 0.1% and 5.5% of camels examined respectively (Mustafa and Nur, 1988). Abu Damir (1984) examined 740 slaughtered camels from Eastern, Western and Central Sudan, and found an incidence of 7.5%, 3.1% and 2% when he respectively used RBPT, SAT and CFT. The overall positive reactors rate was 4.9%. Bitter (1986) in Eastern Sudan, found different prevalence rates in different herds, which ranged
between 16.5% and 32.3% and the overall rate was 26.5% out of 948 animals examined. Bornstein and Musa (1987) examined 102 Sudanese she camels, 5.9% were found positive for brucellosis. Osman and Adlan (1987) found 8% out of 137 camels tested were positive. Tag Elsir (2002) examined 1000 camels in Kassala State, Eastern Sudan and found an incidence of 6%, 8% and 9.1% when he respectively used RBPT, TAT and CTAT.

1.4.5.11.5 The situation of small ruminants brucellosis in the Sudan

*Brucella melitensis* was isolated from cows, sheep and goats' milk in ElGezira, Central Sudan (Dafalla and Khan, 1958). It is also isolated from a ram in an infected flock in Western Sudan (Musa and Jahans, 1990). Serological investigation on the prevalence of the disease was carried by some workers, and the disease was diagnosed in many areas, in Upper Nile Province (Nasri, 1960), in Wad Halfa, Northern Sudan (Abdula, 1966), in Darfur States (Musa, 1995), in Khartoum State (Fayza, Wlsheikh, Zakia, Halim, Suliman and Osman, 1990). Musa (1995) found that the prevalence of brucellosis of sheep and goats in Darfur states was 3.5% and 3.98% respectively. Hayfa (2000) found that Caprine brucellosis in Khartoum state was 0.7%.

**CHAPTER TWO**

**MATERIAL AND METHODS**
2.1 Sterilization

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

2.2 Sources and type of samples

Milk and sera were collected from, cattle camels, sheep and goats. Milk samples from cattle were collected from four dairy farms and milk samples from sheep and goats were collected from small herds owned by nomad or that reared with cattle in farms. Sera from all species were collected from Portsudan abattoir, dairy farms and animals owned by nomads. The sera from the abattoir were collected during antimortem inspection.

A total of 4566 sera samples were collected, 210 sera samples from cattle, 256 from camels, 2000 from sheep and 2000 from goats.

A total of 165 milk samples were also collected, 65 from cattle, 50 from sheep and 50 milk samples from goats.

Sera and milk samples were tested for the presence of antibodies to brucella.

2.3 Collection of samples

2.3.1 Serum samples
Blood samples were collected for sera from cattle, camels, sheep and goats by vena-puncture of the jugular vein using vacutainer tube with needle holder or by using disposable plastic syringe after clipping the hair and disinfecting the skin with alcohol. Samples from the abattoir were collected during antimortem inspection. 5ml blood was collected from each animal. Tubes containing blood samples were placed in racks inside a small ice box on the top of ice and after clotting they were transported to the laboratory, and the sera were separated by centrifugation and placed in small sterile Bijou bottle. The sera were tested in the same day of collection for brucella antibodies using RBPT.

2.3.2 Milk samples

The udder was washed, dried and the tip of the teat was disinfected with alcohol (70%). The first stream of milk was discarded and 10 ml were collected from each animal in sterile universal bottle. Immediately after collection of the milk sample from each animal, the container was labeled and placed into a thermos flask (Alton et al., 1988).

The samples were then transported to the laboratory and refrigerated at 4°C for minimum of 12 hours, because fresh milk testing on the same day of collection may yield false positive reaction, where as such reaction usually disappear after refrigeration, (Brinley-Morgan et al., 1981).

All samples collected were subjected to the milk ring test on the second day of collection.

2.4 Serological test
2.4.1 RBPT

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

The test was done by dispensing 0.03 ml of each serum to be tested to an enamel plated plate and the same amount of RBPT antigen was added to each serum and both were mixed together, rocked by hand for four minutes, after which the test was immediately read. Sera positive to RBPT were classified into five categories:

1. Weak positive: when very weak fine agglutination occurred, this could be hardly seen by unaided eyes.
2. Positives: when the agglutination was fairly visible.
3. Positives with rim formation: when the agglutination appeared prominently in the periphery forming a rim.
4. Strong positives: where there was a granular agglutination.
5. Very strong positive: where the agglutination was very rapid and large clumps occurred, leaving only clear fluid.

2.4.2 MRT

The antigen for the MRT was supplied by the (C.V.R.L), Soba. The antigen was stored in refrigerator at 4°C and used within the expiry date. The milk samples and antigen were removed from the refrigerator and were brought to room temperature before testing.
One ml of thoroughly mixed milk sample was dispensed into agglutination tube to which 0.03 ml antigen was added. The milk sample and the antigen were mixed and incubated at 37°C for 1 hour in an incubator. The results of the MRT were interpreted as follow:

1. 3 Plus positive: the cream layer showed a deep blue ring on top of a completely white column of milk which indicates a high concentration of brucella agglutination.
2. 2 plus positive: the cream layer appeared deep blue colour and the milk column slightly blue.
3. 1 plus positive: both the cream layer and milk column exhibited a blue colour.
4. ± Positive: the cream layer is the colour or slightly more coloured than milk column.
5. Negative: the cream layer is white and the milk column blue.

CHAPTER THREE

RESULTS

3.1 Cattle

A total of 210 cattle sera were examined with RBPT for brucella antibodies, 125 of these sera were from cattle slaughtered in abattoir. 25 (11.9%) of all sera were found positive. Sera collected from the abattoir (125 samples) were from males, and six (4.8%) of them were found positive. 85 sera were
collected from farms and 19 of these sera were positive, 17 from females (22.36%) and 2 from males (22.2%).

65 milk samples were tested with milk ring test, and gave 17 positive reactors. (Table 8 and 9 show the prevalence rates of brucellosis in relation to sex and age).

3.2 Camels

A total of 356 camels' sera were examined for brucellosis and 30 of these sera were from camels slaughtered in abattoir. 68 (19.1%) of all sera were found positive, 15 (19.2%) were females sera and 53 (19.06%) were males. (Table 10 and 11 show prevalence rates according to sex and age).

3.3 Sheep

A total of 2000 sera samples were examined for brucellosis with RBPT and six were found positive (0.3%), and from a total of 50 milk samples tested with MRT one was found positive for brucellosis. (Prevalence rates in relation to sex and age showed in table 12 and 13).

3.4 Goats

A total of 2000 sera were tested with RBPT for brucella antibodies and 9 (0.45%) were found positive. From sera samples which gave positive result, 5 (0.4%) were from males, and 4 (0.53%) were from females.

From a total of 50 milk samples examined with MRT, three were positive for brucellosis. (Table 14 and 15 showed the prevalence rates in relation to sex and age).
Cattle

Table 8: prevalence rate for brucellosis in relation to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. tested</th>
<th>No. positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>134</td>
<td>8</td>
<td>5.9</td>
</tr>
<tr>
<td>Female</td>
<td>76</td>
<td>17</td>
<td>22.36</td>
</tr>
</tbody>
</table>

Table 9: prevalence rate for brucellosis in relation to age
### Table 10: prevalence rate for brucellosis according to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. tested</th>
<th>No. positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>278</td>
<td>53</td>
<td>19.06</td>
</tr>
<tr>
<td>Females</td>
<td>78</td>
<td>15</td>
<td>19.2</td>
</tr>
</tbody>
</table>

### Table 11: prevalence rate for brucellosis in relation to age

<table>
<thead>
<tr>
<th>Age</th>
<th>No. tested</th>
<th>No. positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 years</td>
<td>45</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>3-10 years</td>
<td>157</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>over 10 years</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Age</td>
<td>No. tested</td>
<td>No. positive</td>
<td>%</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>--------------</td>
<td>----</td>
</tr>
<tr>
<td>1-3 years</td>
<td>50</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>3-10 years</td>
<td>106</td>
<td>30</td>
<td>28.2</td>
</tr>
<tr>
<td>10-20 years</td>
<td>200</td>
<td>26</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 12: prevalence rate for brucellosis in according to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. tested</th>
<th>No. positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1400</td>
<td>5</td>
<td>0.35</td>
</tr>
<tr>
<td>Female</td>
<td>600</td>
<td>1</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 13: prevalence rate for brucellosis in according to age
<table>
<thead>
<tr>
<th>Age</th>
<th>No. tested</th>
<th>No. positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1 year</td>
<td>800</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>1-5 years</td>
<td>1200</td>
<td>4</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Goats

Table 14: prevalence rate for brucellosis in relation to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. tested</th>
<th>No. positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1250</td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>Female</td>
<td>750</td>
<td>4</td>
<td>0.53</td>
</tr>
</tbody>
</table>
CHAPTER FOUR

DISCUSSION

The existence of brucellosis in the Sudan for a long time together with lack of eradication programme resulted in a wide spread of the disease.

In country where brucellosis exist, it poses a serious economic problem in domestic animals, causing abortion and decrease in meat, milk, wool production and causing long calving interval and sterility.

In the present study the overall prevalence rates of brucellosis in 210 cattle, 356 camels, 2000 sheep and 2000 goats examined was found to be, 11.9%, 19.1%, 0.3% and 0.45% respectively.

<table>
<thead>
<tr>
<th>Age</th>
<th>No. tested</th>
<th>No. positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-12 months</td>
<td>1300</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>1-5 years</td>
<td>700</td>
<td>5</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Table 15: prevalence rate for brucellosis in relation to age
Many investigators surveyed the prevalence of the disease in different areas of the country; Nasri (1960) in Southern Sudan reported 14% and 18% prevalence rates of the disease in cattle, 6.6% in goats and 35 in sheep. Dafalla (1962) reported prevalence rates 8.7% and 10.7% in cattle, 4.2% and 50% in sheep and 2.5% and 30% in goats, in ElGazera, Central Sudan, and 15% in cattle in Southern Sudan. Mustafa and Hassan (1969) reported 5.7% and 8.7% prevalence rates in nomadic cattle in Blue Nile. Abdalla (1964) in Northern Sudan, reported prevalence rate of brucellosis; 3%, 1.7% and 1.5% for cattle, sheep and goats in villagers animals respectively. Fayza et al., (1990) reported 15.73%, 15.04%, 0.13% and 0.01% prevalence rates in cattle, camels goats and sheep respectively. Musa (1995) reported, 13.9%, 7.76%, 5.98% and 3.52% prevalence rates for cattle, camels, goats and sheep respectively in Darfur States. Mustafa and Awad Elkarim (1971) reported 1.7% prevalence rate of brucellosis in camels. In Eastern Sudan (1986) Bitter found different prevalence rates in different herds of camels, which range between 16.5% and 32.3%, and the overall rate was 26.5% out of 948 camels examined. Osman and Adlan (1987) found 8% out of 137 camels tested were positive in four areas in Eastern Sudan. Yagoub, Mohamed and Salim, (1990) reported 6.95% positive out of 1502 camels examined by RBPT, 4.94% positive out of 1153 adult males, 13.76% out of 270 adult females and one out of 79 young animals. Mustafa and Nur (1968) examined camels in Gash and Toker, reported 1.1% and 5.5% respectively. Raga (2000) reported the prevalence rate, 11.4% for males and 4.2% for she camels in Darfur States.
Cattle

The result of 25 positive (11.9%) out of 210 tested animals is reasonable compared with prevalence rates reported by Dafalla (1962), who reported 8.7% and 10.7% prevalence rate, in ElGazera, Central Sudan, and Fayza et al. (1990) reported 15.73% prevalence rate in Khartoum State, and with that reported by Musa (1995), 13.7% in Darfur States.

The prevalence rate obtained 11.9% is considerably higher compared to that reported by Abdalla (1964), who found overall prevalence rate of brucellosis in cattle in Northern Sudan was 3%.

The prevalence rate in males was (5.9%), and in females was (22.36%). From 134 tested males, 125 were from slaughter house and 6 of these were found positive (4.8%) and from 9 males from dairy farms 2 were found positive (22.2%). The majority of positive animals were from dairy farms, which may be due to bad hygiene and crowdness. Prevalence rate was found to be increased by age and it was 2.2% for animals 1-3 years old, 14% for animals 3-10 years old and 25% for animals over 10 years of age. In old animals frequency of exposure to infection calves can be infected in utero or may acquired infection by consumption of colostrums or milk from infected cow, but a self cure mechanism takes place in most of the infected calves (Grillo, Barberan and Blasco, 1977).

Camels

In the present investigation the overall prevalence of brucellosis in 356 camels was found to be (19.1%), which is comparable to that obtained by Bitter
in the Eastern Sudan, who reported 16.5% and 32.5%, and that reported by Fayza et al. (1990) 15.04%, in Khartoum State. But the prevalence rate (19.1%) obtained is considerably higher compared to that reported by Osman and Adlan, (1987) in Eastern Sudan, 8% and with the prevalence rate reported by Yagoub et al., (1990) in Eastern Sudan, 6.95% and Musa (1995), 7.75% and that found by Raga (2000), 6.2% in Darfur State and Tag ElSir (2002), 6% Kassala State.

Camels are susceptible to Br abortus and Br.meltensis (Nicoletti, 1989); they are infected by lateral infection from the primary host of Br.abortus (cattle), and Br.melitensis (sheep and goats). So the prevalence rate of brucellosis in camels increased when herded with them. Musa (1995) reported 23% prevalence rate in area where camels were reared with cattle, 1.9% and 4.8% in herds newly introduced into such areas. Agab, Abbas, Eljack and Mamon, (1995) isolated Br.abortus from camels serologically positive for brucellosis in Butana area. Zowghi and Ebadi (1988) also isolated Br.melitensis from several cases in camels in Iran.

The prevalence rate found in this study in males (19.06%), and in females (19.2%) agreed with that reported by Musa (1995), 7.05% in males and 7.69% in females, but not with that obtained by Raga (2000) in the same area, Darfur States. She found prevalence rate 11.4% in males and 4.2% in females. Camels 3-10 years old were more infected (28.2%) and camels over 10 years were found less infected (13%). Agab et al., (1995) noticed that the disease increased with the age of 10 years after which the incidence declined.
Sheep and goats

In this study the prevalence rates obtained 0.3% and 0.45% in sheep and goats respectively, agreed with that found by Fayza et al. (1990) in Khartoum State, 0.01% in sheep and 0.13% in goats. ElNasri (1960) reported prevalence rate 6.6% in goats and 3% in sheep in Southern Sudan, and Buamann (1983) did not find any positive cases in sheep and goats examined in Madi-Dinka. Dafalla (1962) found the prevalence rate of the disease between 4.2% to 50% in sheep and 2.5% to 30% in goats in ElGazera, Central Sudan. In Northern Sudan Abdalla (1966) reported 1.7% and 1.5% brucellosis prevalence rates in villager's sheep and goats. In France a strong correlation was found between the prevalence of brucellosis in small ruminants and their movement in pasture (Tylor, Lisbone, Vidal and Hazemann, 1938). Stableforth (1959) mentioned that *brucella abortus* causes sporadic natural infections in sheep and Corbel (1989) added that infections due to this species occasionally occur in sheep as a result of contact with cattle. *Br. abortus* infection in goats were frequently recorded (Corbel, 1989). Musa (1995) mentioned that the incidence of brucellosis in sheep and goats reared with cattle and camels was found to be higher than that ones reared separately, he reported 2.7% to 13% in nomadic sheep herded with cattle and 1.5% to 2.7% in sheep reared separately, 2.4% to 8.5% in nomadic goats reared with cattle and camels and 3.01% in goats reared separately.
In this investigation from the 2000 sheep sera tested with RBPT, 50 milk samples were collected from lactating ewes and tested with MRT. Only one animal was found positive with both RBPT and MRT. The prevalence rate in relation to sex was found, 0.35% in males and 0.17 in females. This result is agreed with that found by Musa (1995), who reported high prevalence rate in males (4.3%), than that in females (3.3%). In relation to age the prevalence rate was 0.25% in sheep less than one year old and 0.33% in animals 105 years old. And from 2000 goats sera tested with RBPT, also 50 milk samples were collected and tested with MRT. Three animals gave positive result with both RBPT and MRT. The prevalence rate in females was 0.53%, and it is higher than that in males (0.4%). The prevalence rate in young animals 6-12 months old was 0.3% and in goats 1-5 years old was 0.71%.

Lambs and kids can be infected in utero, but the majority of infections are probably acquired by consumption of colostrum or milk. It is also probable that self cure mechanism similar to that suggested in cattle takes place in most of infected lambs (Grillo, Barberan and Blasco, 1997), and susceptibility to infection with brucellosis increases after sexual maturity and especially with pregnancy.

Rose Bengal Plate Test is commonly used as screening test for brucellosis in animals and humans as (Brinley-Morgan et al., 1981). It is sensitive and specific test. Diaz, Maravi, Delgado and Rivero, (1978), in their study using RBPT and Counter-Immunoelectrophoresis (CIE) test in the diagnosis of brucella meningitis found that the RBPT was positive in five out of five patients studied.
and CIE was positive in four out of five patients. In comparing a variety of tests used in the serologic diagnosis of human brucellosis RBPT and CIE were found to be better than other tests such as the slide method and immuno diffusion test (Mandell, Douglas, Jr. and Bennett, 1979). Diaz Aparicio, Marin, Alonso, Aragon Perez, Pardo, Blasco, Diaz and Moriyon, 1994, employed five tests for the diagnosis of brucellosis in goats, the tests include: RBPT, CFT, Enzyme-Linked Immunosorbent Assay (ELISA), Radial Immunodiffusion (RID) and Counter Immuno-electrophoresis (CIE). They found that the sensitivity was 100% for RBPT, 94% for CFT and ELISA, and 93% for RID. All tests were 100% specific because they gave negative result when testing sera from brucellosis free goats. Also RBPT is quicker and easier to perform, requires fewer materials and the antigen is less expensive.

RECOMMENDATION
1. More research on the isolation and identification of the species and biovars of the Brucella organisms involved in animal brucella in red sea states

2. Further research on vaccination and suggestion for proper program for eradication of brucella infection.

REFERENCES


Fensterbank, R.; pardon, P.; Marly, J. (1982). Comparison between subcutaneous and conjuntival route of vaccination of Rev.1


Table 1: Differential characteristics of brucella compared to some other gram-negative bacteria

<table>
<thead>
<tr>
<th>Test</th>
<th>Brucella</th>
<th>Bordetella bronchi-septica</th>
<th>Campylobacter fetus</th>
<th>Moraxella</th>
<th>Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Small coccobacilli</td>
<td>Small coccobacilli</td>
<td>Comma</td>
<td>Diplococcoid</td>
<td>D</td>
</tr>
<tr>
<td>Motility at 37°C</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>V</td>
</tr>
<tr>
<td>Motility at 20°C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
</tr>
<tr>
<td>Lactose fermentation on macConkey agar</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
</tr>
<tr>
<td>Acid production on agar containing lactose</td>
<td>-b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
</tr>
<tr>
<td>Haemolysis on blood agar</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+c</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>Urease</td>
<td>+d</td>
<td>+</td>
<td>-</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+c</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Agglutination with:S-brucella antiserum</td>
<td>+f</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-brucella antiserum</td>
<td>+g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a= positive and negative species within the genus.  
b= Br.neotomae may show fermentation. 
c= except Br. Ovis, Br.neotomae and some strains of Br.abortus ,  
d= except Br.ovis and some strains of other species.  
f=except Br.ovis, Br.canis and R-forms of other species.  
g= Br.ovis, Br.canis and R-forms of other species.