1. INTRODUCTION

1.1. Overview:

Animal and human health's are inextricably linked. People depend on animals for nutrition, socioeconomic development and companionship. Yet animals can transmit many diseases to humans. Diseases transmitted from animals to humans are termed zoonosis. Some of them are potentially devastating.

Brucellosis is the zoonosis of worldwide distribution and common cause of economic losses and ill health among animals and human populations (Bennet ., 1943).

Brucellosis is an infectious zoonotic disease caused by bacteria of the genus *Brucella*. It is primarily a disease of domestic animals. Various *BrucelIa* species affect sheep, goats, cattle, deer, elk, pigs, dogs and several other vertebrates. Humans become infected by coming in contact with infected animals or animal products (mostely raw milk and its derivitis). Only in exceptional circumstances transmission can be from man to man (e.g., unsuspected brucellosis in blood donors may lead to infection of recipients, or from lady to her child during breast-feeding). Occasionally, brucellosis is acquired directly from contaminated

laboratory materials (Zammit, 1984).

Some vaccines were used in livestock, most notably was *B. abortus* strain 19, which may cause disease in human if accidently infected. The disease in humans can cause a range of symptoms like those associated with many other febrile diseases, but with emphasis on muscular pain and sweating. Severe infections of the central nervous system or lining of the

heart may occur. Brucellosis also cause long-lasting or chronic symptoms that include reclessent fevers, joint pains, orchitis, meningitis and fatigue (CDC, 2005).In human mortality is negligible, but the illness can last for several years (Madkour.,2001). In animals, brucellosis mainly affects reproduction and fertility, reduces survival of newborns and reduces milk yield, but mortality adult animals is insignificant (Sewell and Brocklesby., 1990). *Brucella* species known are *Brulcella abortus* cattlearethemain reservoir), *B. suis* (pigs, hares, rodents and reindeers), *B. canis* (dogs), *B. avis* (sheep), *B. neotomae* (wood rat neotomae lepida Thomas). *Brulcella* species have also been isolated from several marine mammal species; *B. cetaceae*" preferentially infecting cetaceans" and *B. pinnipediae* " preferentially infecting pinnipeds" (Cloeckaert and zygmunt .,2001). In all host species brucella grow intercellularly producing a variable bacteraemic phases followed by localization in the tissues o the genital tract and in the mammary glands and in humans mainly in the reticuloendothelial system (Jinkyung and Gary, 2003). Pathological manifestations as abortion, placentitis, endometerities, orchaitis, hygromas, epidedymitis and arthritis are not uncommon sequellae of infection with brucellosis in animals (Blood *et al.*, 1983). Bruccellosis is diagnosed either by isolation of Brucella organism organism or by a combination of serological tests and clinical findings consistent with brucellosis (AI Sekait ., 1999). The main way of preventing brucellosis is by control of animal brucellosis by using vaccines, also by using fastidious hygiene in producing raw milk products. In many developed countries, the animals diseases have been brought under control, which has led to subsequent decrease in the number of human cases(WHO.,2008).

1.2 Rationale:

Brulcellosis is a worldwide zoonotic disease. The absence of recent epidemiological data on human and animal. brucellosis as well as the inexistence of an ongoing control program for the disease necessitated the conduction of epidemiological studies that aim at providing useful data for further studies and/ or intervention policies.

In many countries around the world laboratory-acquired Brulcellosis, and abattoirs workers infected by brucellae species has been reported. Prevalence rate among abattoirs workers in Spain was 3.1% (Villamarin-vazquez , *et al.*, 2002) and in Nigeria was 31.82% (Cadmus *et al.*, 2006).

In the United States a review of laboratory-associated infections during 1979 - 1999 revealed that *Brucella* species accounted for approximately 8% of all laboratory infections, 16% of bacterial infections, and 4% of death (Harding and Byers., 2000). A survey on human brucellosis was carried out showed 10% of the disease among workers in Umdurman house, Khartoum State; but there is no report about -acquired brucellosis in the Sudan (Suliman., 2006). Seroprevalence of brucellosis among blood donors carried out in india [Karnataka city] showed 5.91% (Cadmus *et al*., 2006).

1.3 Objectives:

1.3.1. Main objective:

To determine the prevalence of human brucellosis among donors in Khartoum state.

1.3.2. Specific objectives:

1) To determine the prevalence of anti-Brucella antibodies by using Rose Bengal PlateTest (RBpt) and the Standard Agglutination Test (SAT).

2) To determine *Brucella abortus* and *Brucella meletensis* antibody titers wthin blood donors.

2. LITERATURE REVIEW

2.1. History:

Brulcella was isolated in 1886 by David Bruce, an army doctor serving with the British army on the island of Malta. He isolated the organisms from the spleen of four British soldiers dying on the island of Malta from a disease known as Malta fever, or Mediterranean remittent fever (Greenwood *et al*., 2000).

At that time, the disease had a high prevalence among the army and navy personnel and among the islands civilian population.

The name *Brulcella* was subsequently given in honour of Bruce who established it as the cause of the disease in experimental monkeys (Greenwood *et al.,* 2000).

2.1.1. Human brucellosis in Sudan:

Nomads and occupational, namely veterinary staff, abattoir and butchers-house workers were found to be most affected with the disease (Mus a, 1995). The disease was diagnosed in humans in Berber in the Sudan since 1904 (Haseeb.,1950). In 1908, Bousefield reported a case of Malta fever. In the same year 20 cases were reported (Simpson.,1908), 19 of which were clinically diagnosed at Roseires (Blue Nile Province) and one at Kassala. The data given by (Haseeb.,1950) between the year 1925 and 1942 gave a total record of 920 human cases with occurrence in everyone of the eighteen provinces. Medical reports between the year 1928 and 1937 showed the occurrence of 311 human cases and the distribution of the disease was reported from all the nine provinces of the Sudan (Dafaalla.,1962). The organism was isolated from man (Erawa.,1966). In 1982, the Sudan medical reports documented a 242 cases of human

brucellosis. In 1994, AL- Sharif obtained positive results from abattoir workers in Umdurman city slaughter house. In a country where hospital services, particularly where animal abide, is scarce and where fever is "just. a fever" its highly likely that the difference between the actual incidence and the recorded one may be highly significant (Adil.,2007).

2.1.1.2 The disease in humans:

Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months. Typically, few objective signs are apparent but enlargement of the liver, spleen and/or lymph nodes may occur, as many signs referable to almost

any other organ system. The acute phase may progress to a chronic one with relapse, development of persistent localized

infection or a non-specific syndrome resembling the “chronic fatigue syndrome”. The disease is always caused by infection with a *Brucella* strain and diagnosis must be supported by laboratory tests which indicate the presence of the organism or a specific immune response to its antigens. (Ariza *et al.,* 2007)

Evidence in support of the diagnosis includes:

• A history of recent exposure to a known or probable source of *Brucella* spp.

This includes common host species, especially cattle, sheep, goats, pigs, camels, yaks, buffaloes or dogs; consumption of raw or inadequately cooked milk or milk products, and, to a lesser extent, meat and offal

derived from these animals. In addition, the resistance of the organism and its high infectivity make environmental contamination a probable hazard, although this is always difficult to prove. Occupational exposure and/or residence in an area in which the infection is prevalent, also raise the probability of the diagnosis. (Ariza *et al.,* 2007)

• Isolation of *Brucella* spp. from the patient.

• Demonstration by validated polymerase chain reaction (PCR) of the presence of *Brucella* genetic material in blood or other tissue sample(WHO., 2008).

• Demonstration by a validated serological method of *Brucella* antigen in blood or other tissue sample.

• Demonstration of a rising antibody titre in any serological test for brucellosis in the absence of exposure to any known source of cross-reacting antigens(WHO., 2008).

• Demonstration of a high sustained IgG antibody titre in the agglutination, complement fixation or ELISA tests with standardized antigens. (Ariza *et al.,* 2007)

Susceptibility to brucellosis in humans depends on various factors, including the immune status, routes of infection, size of the inoculum and, to some extent, the species of *Brucella*. In general, *B. melitensis* and *B. suis* are more virulent for humans than *B. abortus* and *B. canis*, although serious complications can occur with any species of *Brucella*. (Ariza *et al.,* 2007)

Common routes of infection include direct inoculation through cuts and abrasions in the skin, inoculation via the conjunctival sac of the eyes,

inhalation of infectious aerosols, and ingestion of infectious unpasteurized milk or other dairy products. Blood transfusion, tissue transplantation and sexual transmission are possible but rare routes of infection. (Ariza *et al.,* 2007)

The disease is acute in about half the cases, with an incubation period of two to three weeks. In the other half, the onset is insidious, with signs and symptoms developing over a period of weeks to months from the infection. (WHO., 2008)

The clinical manifestations are varied and nonspecific. They include fever, sweats, fatigue, malaise, anorexia, weight loss, headache, arthralgia and back pain. Commonly, patients feel better in the morning, with symptomsworsening as the day progresses. The desire to rest can be profound, and depression is pervasive. If untreated, the pattern of the fever waxes and wanes over several days ("undulant fever"). (WHO., 2008)

Brucella species are facultative intracellular pathogens that can survive and multiply within phagocytic cells of the host. The mechanisms by which *Brucella* evades intracellular killing are incompletely understood. Nevertheless, *Brucella* organisms ultimately become sequestered within monocytes and macrophages of the reticuloendothelial system (RES), such as lymph nodes, liver, spleen and bone marrow. Brucellosis is a systemic infection that can involve any organ or tissue of the body. When clinical symptoms related to a specific organ predominate, the disease is termed "localized" . (WHO.,2008).

Although humoral antibodies appear to play some role in resistance to infection, the principal mechanism of recovery from brucellosis is cell-mediated. Cellular immunity involves the development of specific

cytotoxic T lymphocytes and activation of macrophages, enhancing their bactericidal activity, through the release of cytokines (e.g. gamma interferon and tumour necrosis factor) from specifically committed helper T lymphocytes. Coincident with the development of cell-mediated immunity, the host usually demonstrates dermal delayed type hypersensitivity to antigens of *Brucella*. (Banai *et al., 2007*)

2.2 Morphology:

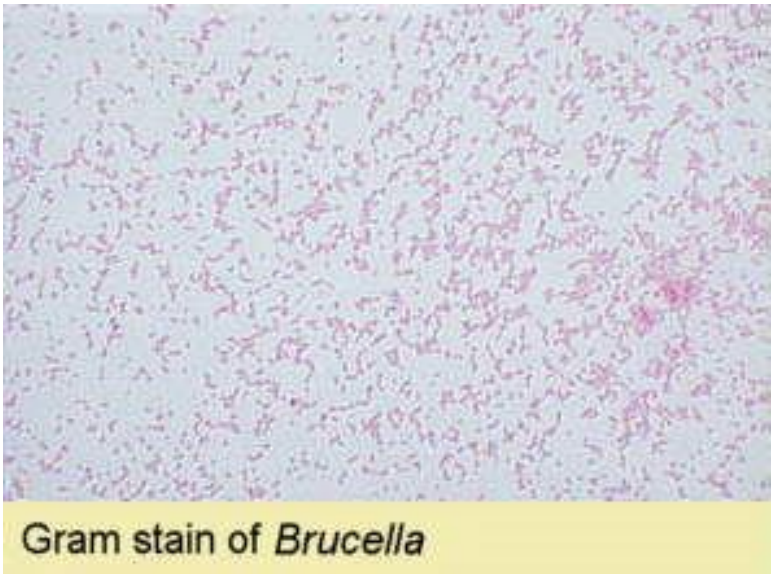

Figure 1.1 Gram negative coccobacilli of *Brucella spp*

Typically Brucella spp. occurs as small Gram-negative coccobacilli,

but coccal and bacillary forms may also occur. The cells are short and slender, the axis is straight; the ends are rounded; the sides may be parallel or convex outwards (Corbel, 1998).

In length they vary from about 0.6µm to 1.5 µm, and in breadth from 0.5 µm to 0.7 µm. The short forms may appear as oval cocci, or if they are arranged singly, in pairs end to end, or in small groups; sometimes short chains of 4-6 members may be seen, especially in liquid media. Because

at their frequently coccoid appearance, their bacillary nature may be in doubt but it may be noted that they are smaller than any of the Gram-negative bacilli. Moreover, when arranged in pairs, their long diameter is in the same axis as that in which they are lying, as distinct from the gram-negative diplococci, whose long axis is generally at right angles to that in which they are lying. *B. melitensis* tends to be more coccal in form than *B. abortus* but this is not consistent enough to be of value for identification. The bacillary forms of *B. abortus* and *B. suis* are most readily apparent when grown on a rich medium, in which individual cells may reach 2-3 µm in length. *B. melitensis* usually remains cocoid and rarely exceeds I µm in length. The organisms stain fairly well with the ordinary dyes. They are Gram-negative, nonacid fast, non-motile and non-sporing. Bipolar staining can occur and irregularity in the depth of colour may be seen especially in old cultures in which irregular forms appear (Corbel., 1998).

Brucella cells resist decolorization by dilute solutions of acids and alkalis, an advantage that has been utilized in a differential staining procedure known as Modified Z.N methods (Stamp *et al.*, 1950).

2.3 Classification:

The genus *Brucella* comprises a group of closely related species. Molecular genetic studies have indicated that the genus contains only a single species differentiated into a number of biovars, with certain host preferences (Corbel., 1998).

The taxonomic validity of this viewpoint has been accepted but the proposed new nomenclature, which would identify all members of the genus as biovars of *B. melitensis*, has been met with opposition on

practical ground (Corbel.,1998). According to Jinkyung and Gary, (2003), currently there are seven nomen species classified as follows:

2.3.1. *B. melitensis:*

It is the first member of the group, and was isolated by Bruce in 1887 from spleen tissue of patients died of Malta fever. Because of its cocoid morphology in vivo and on primary culture in vitro, the organism was described as a micrococcus. The name Micrococcus *melitensis* appeared earlier. Sheep and goats are the preferred natural hosts of *B. melitensis*, but other animals may also be infected. As with other member of the genus, *B. melitensis* tends to localize in the reticuloendothelial system and the genital tract; genital infection of the pregnant female typically results in abortion.

Humans are susceptible to infection, often manifest initially as an acute febrile illness that have been described by various names including Malta fever, Mediterranean fever, and undulant fever. Chronic complications may succeed the acute phase and the term brucellosis is used as a convenient description for all phases of the disease (Adil ., 2007).

2.3.2. *Brucella abortus:*

It is the second member of the group, and was discovered by Bang in 1897, who isolated the organism from cows with contagious abortion, and by a series of experiments demonstrated its specific role in this disease. Cattle are the preferred natural host of this organism but it can also infect other animals. Although usually less virulent than *B. melitensis*, it can also cause brucellosis in humans.

The relationship between *B. abortus* and *B. melitensis* was not appreciated until attention was drawn by their similarities by Evans

(1914). This led Meyer and Shaw (1920) to propose the genus *Brucella* to include both organisms.

2.3.3. *Brucella suis:*

It was reported as the third member of the genus *Brulcella*. It was first reported in 1914 by Traum, who reported its isolation from a fetus of a cow. It is a natural parasite of pigs, frequently producing a generalized infection but with a tendency to localize in the genital tract. Infection can also be transmitted to other animals, although the host range tends to be narrower than that of *B. abortus* and *B.*; *melitensis* possibly for geographical rather than biological reasons. In contrast with the *B. suis* strains described by Traum (1914), other types have been found which showed a different range and pathogenicity. Thus, Thomsen (1934) isolated from pigs in Denmark, strains that differed in certain cultural properties from those found in the USA, and were less pathogenic for humans. These Danish or European porcine strains have hares and swine as their natural hosts, and have been classified as *B. suis* biogroup (biovar) 2. The American strains have been assigned to B. suis biogroups 1 and 3. Two additional B. suis biogroups have also been defined, neither of which cause natural infection in swine. *B. suis* biogroup 4 described earlier as a separate species " *B. rangiferi tarandi*" was associated with brucellosis in reindeer in Alaska, Canada, and Northern Russia, and causing reproductive failure (Davydov, 1961). It is transmissible to humans and causes an undulant-fever syndrome. *B. suis* biogroup 5 has only been found in mouse-like rodents in the Caucasus. However, it is known to be pathogenic for humans and causes a disease similar to that produced by the other *B. suis* biogroups. Generally, *B.suis* strains are less widely distributed geographically than *B. abortus* or *B. melitensis.* (Vershilova and Liamkin., 1983)

2.3.4. *Brucella ovis*:

The fourth member of the Brucella group, *B. ovis* was first observed at about the same time in Australia and New Zealand (BuddIe and Boyes, 1953) and identified as the cause of epididymitis in rams. It has since been found in most other sheep-raising countries including Argentina, Chile, France, Germany, South Africa, USA, Spain and countries of the former Soviet Union. Although serological evidence suggests that human can be infected by this organism, it has not been confirmed to be a cause of overt disease.

2.3.5. *Brucella neotomae:*

The fifth member, *B. neotomae*, was isolated from desert wood rats in USA. It has not been associated with disease in humans or other species and further isolates have not been recorded.

2.3.6. *Brucella canis:*

The sixth member of the genus, *B. canis*, was reported by Carmichael and Bruner in 1968 (Adil, 2007) as the cause of abortion in beagle dogs in the USA. It has since been found in dogs of various breeds in many countries. Occasional cases have also been reported In humans, usually presenting as a mild pyrexial, illness. Unlike *B. abortus*, *B. melitensis* and *B. suis*, in which virulence is associated with the smooth colonial form, *B. canus* and B. ovis have been found only in the smooth phase. This has implications for the serological diagnosis of infections caused by these organisms because smooth and non-smooth brucella strains are antigenically distinct (Adil., 2007).

2.3.7. *Brucella mans:*

Recently, *Brucella mans* strain has been isolated from marine mammals (Ross *et al.*, 1994). The isolates comprise at least two distinct biogroups corresponding to strains of cetacean and phocine origin. Within these groups there is some variations in metabolic and antigenic properties. However, it is apparent that these isolates differ from the *Brucella* strains infecting terrestrial mammals. They appear to have low pathogenicity for ruminants but circumstantial evidence suggests that they are pathogenic for humans (Ross *et al*.,1994).

2.3.7.1 Clinical manifestations:

Brucellosis is essentially a disease of animals, especially domesticated livestock, caused by bacteria of the *Brucella* group with humans as an accidental host. In other words it is a zoonosis. On genetic grounds the *Brucella* group can be regarded as variants of a single species which for historical reasons is identified as *Brucella melitensis.* However, for practical purposes this approach is considered unsatisfactory and six main "species" are distinguished: (Banai *et al., 2007)*

B. abortus, B. suis, B. melitensis, B. neotomae, B. ovis, B. canis.

Strains isolated from marine mammals fall into at least three groups distinct from these and may be designated as new "nomen species". (Banai *et al., 2007)*

The differentiation of these variants is of practical importance as the epidemiology and, to a lesser extent, the severity of the disease in humans, is influenced by the type of organism and its source. Thus *B. abortus* is normally associated with cattle, *B. melitensis* with sheep and goats, *B. suis* with swine (although biovars 4 and 5 are specifically

associated with reindeer and rodents respectively). *B. ovis* causes an infection specific for sheep and has not been conclusively implicated in human disease, *B. suis* biovar 5 has only been isolated on a few occasions from rodents and *B. canis* is usually associated with disease in dogs but occasionally causes human brucellosis. *B. neotomae* has been isolated on few occasions and has never been implicated in human disease. (Banai *et al., 2007)*

The human disease usually manifests itself as an acute febrile illness which may persist and progress to a chronically incapacitating disease with severe complications. It is nearly always acquired directly or indirectly from animal sources, of which cattle, sheep, goats and pigs are by far the most important.

In these natural hosts, the infection usually establishes itself in the reproductive tract, often resulting in abortion. Excretion in genital discharges and milk is common and is a major source of human infection. (Banai *et al., 2007)*

The clinical picture is not specific in animals or humans and diagnosis needs to be supported by laboratory tests. Effective treatment is available for the human disease but prevention is the ideal, through control of the infection in animals and by implementation of hygienic measures at the individual and public health levels. (Ariza *et al.,* 2007)

2.3.7.2 Osteoarticular complications

Bone and joint involvement are the most frequent complications of brucellosis, occurring in up to 40% of cases. A variety of syndromes have been reported, including sacroiliitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis, and tenosynovitis. *Brucella* sacroiliitis is

especially common. Patients present with fever and back pain, often radiating down the legs (sciatica). Children may refuse to walk and bear weight on an extremity. Early in the disease, radiographs and bone scintigrams can appear normal, but, in time, computed tomography (CT) or nuclear magnetic resonance (NMR) scans may show narrowing of the intervertebral disc space. Vertebral osteomyelitis is readily apparent through radionucleide scans showing destruction of the vertebral bodies. The lumbar vertebrae are involved more often than the thoracic and cervical spine. Paravertebral abscesses are less common in brucellosis than in spinal tuberculosis. A post-infectious spondyloarthropathy involving multiple joints has been described, and is believed to be caused by circulating immune complexes.(Banai *et al., 2007*)

2.4 Gastrointestinal complications

Brucellosis, especially when due to *B. melitensis,* is often food borne, and unpasteurized milk or dairy products, such as cheese, are common vehicles of transmission. Food borne brucellosis resembles typhoid fever, in that systemic symptoms predominate over gastrointestinal complaints. Nevertheless, some patients with the disease experience nausea, vomiting, and abdominal discomfort. Rare cases of ileitis, colitis and spontaneous bacterial peritonitis have been reported. (Ariza *et al.,* 2007)

2.4.1 Hepatobiliary complications

The liver is commonly involved in brucellosis, although liver function tests can be normal or only mildly elevated. The histological changes in the liver are variable, but disease caused by *B. abortus* may show epithelioid granulomas that are indistinguishable from sarcoidosis lesions. A spectrum of hepatic

lesions has been described in cases due to *B. melitensis,* including scattered small foci of inflammation resembling viral hepatitis. Occasionally larger aggregates of inflammatory cells are found within the liver parenchyma with areas of hepatocellular necrosis. In other cases, small, loosely formed epithelioid granulomas with giant cells can be found(Banai *et al., 2007).*

Despite the extent of hepatic involvement, post-necrotic cirrhosis is extremely rare. Hepatic abscesses and chronic suppurative lesions of the liver and other organs have been described in cases due to *B. suis.* Acute and chronic cholecystitis have been reported in association with brucellosis. (Banai *et al., 2007*).

2.4.2 Respiratory tract complications

Aerosol inhalation is a recognized route of transmission of brucellosis, especially common in abattoirs where infected animals are slaughtered. A variety of pulmonary complications have been reported, including hilar and paratracheal lymphadenopathy, interstitial pneumonitis, bronchopneumonia, lung nodules, pleural effusions, and empyema. *Brucella* organisms are rarely isolated from expectorated sputum. (Ariza *et al.,* 2007).

2.4.3 Genitourinary complications

Orchitis and epididymitis are the most frequent genitourinary complications of brucellosis in men. Usually unilateral, *Brucella* orchitis can mimic testicular cancer or tuberculosis. Although *Brucella* organisms have been recovered from banked human spermatozoa, there have been a few reports implicating sexual transmission. Renal involvement in brucellosis is rare, but it too can resemble renal tuberculosis. In women,

rare cases of pelvic abscesses and salpingitis have been reported.(Banai *et al., 2007).*

2.4.4 Pregnancy and breastfeeding

Brucellosis during the course of pregnancy carries the risk of spontaneous abortion or intrauterine transmission to the infant. Abortion is a frequent complication of brucellosis in animals, where placental localization is believed to be associated with erythritol, a growth stimulant for *B. abortus.* Although erythritol is not present in human placental tissue, *Brucella* bacteremia can result in abortion, especially during the early trimesters. Whether the rate of abortions from brucellosis exceeds rates associated with bacteremia from other bacterial causes is unclear. In any event, prompt diagnosis and treatment of brucellosis during pregnancy can be lifesaving for the fetus. (Banai *et al., 2007*).

Very rare human-to-human transmission from lactating mothers to their breastfed infants has been reported. (Ariza *et al.,* 2007).

2.4.5 Cardiovascular complications

Infective endocarditis is the most common cardiovascular manifestation, and it is said to be the most common cause of death from brucellosis. Endocarditis is reported in about 2% of cases, and can involve both native and prosthetic heart valves. The aortic valve is involved more often than the mitral valve. (Banai *et al., 2007*).

Aneurysms of the sinus of Valsalva and other vascular structures appear to be most common when infection is caused by *B. suis.* Mycotic aneurysms, usually involving the middle cerebral artery, can be a neurological complication of infective endocarditis. Treatment of

endocarditis caused by *Brucella* species usually requires a combination of antimicrobial therapy and valve replacement surgery. (WHO; 2008)

2.4.6 Neurological complications

Neurobrucellosis refers to a variety of neurological complications associated with brucellosis. Direct invasion of the central nervous system occurs in about 5% of cases of *B. melitensis* infection, and meningitis or meningoencephalitis are the most common manifestations. *Brucella* meningitis can be acute or chronic. It often occurs late in the course of disease, but it can be the presenting manifestation. Analysis of cerebrospinal fluid (CSF) usually reveals an elevated protein content, normal or low glucose concentration,

and a lymphocytic pleocytosis. *Brucella* organisms are rarely isolated from CSF, but specific antibodies can be demonstrated in the CSF and serum. (Banai *et al., 2007).*

Other Central Nervou System {CNS} manifestations of brucellosis include cerebral vasculitis, mycotic aneurysms, brain and epidural abscesses, infarcts, haemorrhage, and cerebellar ataxia. Peripheral nerve complications include neuropathy/radiculopathy, Guillain-Barré syndrome, and a poliomyelitis-like syndrome. (Banai *et al., 2007*).

Brain scans (e.g. CT, magnetic resonance imaging) are usually normal in meningitis, but can be useful for detecting space-occupying lesions and the integrity of the epidural space. Basal ganglia calcification has been reported in some patients with neuro-brucellosis. (Ariza *et al.,* 2007).

2.4.7 Cutaneous complications

A variety of skin lesions have been reported in patients with brucellosis, including rashes, nodules, papules, erythema nodosum, petechiae, and purpura. (Banai *et al., 2007*).

Cutaneous ulcers, abscesses, and suppurative lymphangitis appear to be more common with *B. suis.* Occasionally, epistaxis, gingivorrhea, haematuria, and cutaneous purpura occur in association with severe thrombocytopenia, which has been ascribed to hypersplenism, bone marrow haemaphagocytosis, and/or anti-platelet antibodies. (Banai *et al., 2007).*

2.4.8 Opthalmic complications

Although uncommon, a variety of ocular lesions have been reported in patients with brucellosis. Uveitis is the most frequent manifestation, and can present as chronic iridocyclitis, nummular keratitis, multifocal choroiditis or optic neuritis. Since *Brucella* organisms have not been isolated from the structures of the eye in humans, many of these lesions are considered to be late complications, possibly immunologically mediated. Consequently, the usual treatment for ocular complications is steroids. (Ariza *et al.*, 2007).

2.4.9 Chronic brucellosis:

Perhaps no aspect of the disease elicits more controversy than chronic brucellosis. This is due, in part, to the lack of a universally accepted definition. (Banai *et al., 2007*).

Most authorities agree that the term “chronic brucellosis” should be reserved for patients whose clinical symptoms persist for 12 months or

more from the time of the diagnosis. Using this criterion, patients fall into three categories:

(1) relapse, (2) chronic localized infection, and (3) delayed convalescence. (Banai *et al., 2007*).

Relapse is defined as the recurrence of characteristic signs and symptoms (with or without a positive culture) occurring at some time after the completion of a course of treatment. Patients with relapse characteristically have objective signs of infection, such as fever, and persistently elevated titres of IgG antibodies in their serum. Most relapses occur within six months after therapy is discontinued, and relapse is not usually due to the emergence of antibiotic resistant strains, although this has been seen after monotherapy with rifampicin or streptomycin. (Banai *et al., 2007).*

Therefore, relapse can usually be treated by repeating the course of therapy with the same drugs. (Ariza *et al.,* 2007).

Chronic localized infection is defined as the recurrence of characteristic signs and symptoms (with or without a positive blood culture) caused by the failure to eliminate a deep focus of infection, such as osteomyelitis, or deep tissue abscesses. Patients with localized infection have also objective signs of infection, such as fever, although symptoms may recur intermittently over long periods of time. As is the case with patients with relapse, localized

infection is characterized by persistent elevation of IgG antibodies in the serum. Unlike relapse, chronic localized brucellosis may require surgical intervention to drain foci of infection in addition to antimicrobial therapy. (Ariza *et al.,* 2007).

Delayed convalescence is defined as the persistence of symptoms, without objective signs of infection, such as fever, in patients who have completed a course of therapy, and in whom titres of antibodies have declined or even disappeared. The etiology of delayed convalescence is unknown, but psychological

studies of some patients suggest a high incidence of personality disorders, often predating the onset of brucellosis. In any case, patients with delayed convalescence do not appear to benefit from repeated courses of antimicrobial therapy. (Banai *et al., 2007).*

2.4.10 Childhood brucellosis

Once considered rare in children, it is now recognized that brucellosis can affect persons of all ages, especially in areas where *B. melitensis* is the predominant species. The course of infection and the incidence of complications appear to be similar regardless of the age of the patients. (Ariza *et al.,* 2007).

KEY POINTS ON THE DISEASE IN HUMANS

• Human brucellosis usually presents as an acute febrile illness.

• Most cases are caused by *B. melitensis.*

• All age groups are affected.

• Complications may affect any organ system.

• The disease may persist as relapse, chronic localized infection

or delayed convalescence.

2.5. Cultural characteristics:

A part from different carbon dioxide requirement, all members of the genus resemble each other closely in cultural properties. Although most strains will grow on nutrient agar, growth tends to be slow and colony size is small (Corbel and Hendry, 1985).

Growth is much improved by the addition of blood, serum and tissue extracts. Liver infusion agar was formerly recommended for the isolation of *Brucella spp*. But has been superseded by more consistent media based on high quality peptone preparation, usually tryptic digests of soybeans protein. These will support the growth of all but the most fastidious strains. On serum dextrose agar or similar medium, most *Brucella* strains produce raised, convex, circular, translucent colonies, 0.5 - 1 mm in diameter, after incubatiun for 48 hours at 37°C. In direct light the colonies of smooth strains show a clear honey colour, but in reflected light they have a distinctive bluish-grey translucency. Nonsmooth strains produce colonies of similar size but of much more variable colour and consistency. Strains of less fastidious types, especially *B. melitensis* and *B. suis* will grow on bile-salt media producing small lactose-negative colonies (Corbel and Hendry, 1985).

Growth in gelatin is poor and liquefaction is not produced. On blood agar the colonial appearance is not distinctive, true haemolysis is not produced but greenish brown discoloration develops around the colonies. In static liquid culture, maximum turbidity is produced after 7 days or longer. Most strains produce a uniform turbidity with variable deposits. Smooth colonies appear small, circular, convex, translucent and grayish-blue with a smooth glistering surface. The individual cells are uniformly short rods or cocco-bacilli arranged singly. Colonies of rough strains are of much

the same size, but are less convex, more opaque and have a dull dry yellowish-white granular appearance (Corbel and Hendry, 1985).

The individual cells are rather larger than those of smooth form, and occasional long slender rods are present (Corbel and Hendry, 1985).

2.6. Metabolism and biochemical properties:

All *Brucella* strains are aerobic and require oxygen for growth. No growth occurs under strictly anaerobic conditions. Some strains require supplementary carbon dioxide. Although acid from sugars has been demonstrated under special conditions, metabolism is essentially oxidative (Pikett and Nelson, 1955). *Brucella* strains are catalase positive and most are oxidase positive. They also have superoxide dismutase activity mediated by 2 distinct enzymes, a manganese superoxide dismutase of typical prokaryote type (Sriranganath and Boyle, 1991), and copper-zinc enzyme (Beck *et al*, 1990).

Energy yielding processes Involve the mono phosphate pathway and tricarboxylic acid cycle (Roberson and McCullough, 1964). Glucose IS metabolized by most strains but isoerythritol is used preferentially by *B. abortus*, *B. melitensis* and *B. suis. B. ovis* shows little activity towards either substrates; its energy source is unknown, but it has been shown to oxidize water-soluble components of various ovine tissues very actively (Redwood and

Corbel 1983).

Most *Brucella* strains produce nitrate reductase and will reduce nitrate to nitrite and also reduce nitrite, especially *B. suis* Biogroup 1. *B. avis* and, occasionally, strains of other species, do not reduce nitrate. Hydrogen sulphide is produced from sulphur-containing amino acids. Strains of *B.*

abortus, *B. neotomae* and *B. suis* biogroup 1 produce it in sufficient quantities to assist in their identification; the other species produce it in small quantities or not at all. *Brucella* strains do not produce indole from tryptophan. Urease is produced by most strains but the activity varies considerably between species and even between strains within species. *B. canis* and *B. suis* consistently give strong urease reaction, producing magenta colour on Christensen's medium within 5 minutes. At the other extreme, *B. ovis* is a weak producer of urease and some strains may give negative reaction even after incubation for 7 days. Strains of *B. abortus*, *B. melitensis* and *B. neotomae* fall between these extremes and usually give positive urease reaction after one hour or more. Nevertheless variation occurs between strains and the test is of limited value for species identification (Corbel and Hendry, 1985). The oxidative activity of *Brucella* strains towards selected amino acid and carbohydrate substrates varies in a manner which correlates closely with other properties used to define the species. Originally, manometric methods were used to detect oxidation and although hazardous and time-consuming to perform, they are still useful when quantitative studies are to be performed. Nevertheless, quantitative variations in activity between strains within species can cause confusion (Corbel and Hendry, 1985). To overcome this problem, Verger and Grayon (1977) established three levels of oxidative activity, corresponding to low, medium and high oxygen uptake, which they used to produce a metabolic profile for each of the main species. The metabolic activity towards selected substrates can also be determined qualitatively by thin layer chromatography (Balke *et aI.*,1977).

The patterns of oxidative activity characteristic of species are shown in Table 2-1.

Table 2-1: Oxidative metabolism of species of *Brucella.*

	B. melitensis	***B. abortus***	***B. suis***	***B. neotomae***	***B. canis***	***B. ovis***
Aminoacids L-Alanine	+	+	v*	V	v	v
L-Aspargine	+	+	v*	+	-	+
L-Glutamate	-	+	v*	+	+	+
L-Arginine	-	-	+	-	+	-
Aminoacids	*B. melitensis*	*B. abortus*	*B. suis*	*B. neotomae*	*B. canis*	*B. ovis*
DL-Citrulline	-	-	+	-	+-	-
L-Lysine	-	-	v*	-	+	-
DL-Omithine	-	-	+	-	+	-
Carbohydrates L-Arabinose		+	v*	-	+	-
D-Galactose	-	+	v*	+	v	-
D-Ribose	-	+	+	V	+	-
D-Xylose	-	v	-	-	-	-
D-Glucose	+	+	+	+	+	-
Isoerythritol	+	+	+	+	v	-

+, Positive; -, negative; v, variation between strains; v*, variation between biogroups of some assistance in classification (Corbel, 1998).

2.7. Antigenic structure:

The smooth lipopolysaccharide (S-LPS) is the immunodominant antigen of the *Brucella* cell surface and major virulence factor. It has endotoxic activity but shows some major differences in activity from enteric endotoxins typified by *E. coli* LPS. For example it is much less toxic for rabbits, chick embryos and endotoxin-sensitive mouse strains. It is much more toxic than E.eoti endotoxin for endotoxin-resistant mouse strains and is effective in stimulating interleukin-1 and tumor necrosis factor-a (TNF-a). It is also toxic for macrophages and is antigenic for spleen B but not T-Lymphocytes (Cloeckaert and Zygmunt, 1992).

Its lipid A does not bind polymyxin B and this does not necessarily inhibit its mitogenic or toxic activity. *Brucella* LPS has an unusual adjuvant activity in that it stimulates high level of IgG and IgM antibodies in mice. It's a major protective antigen in mice and other species including cattle (Corbel., 1998). Various polysaccharides structurally related to *Brucella* LPS a chain have been described. These include 'native antigen' or 'native hapten'. This can be extracted from LPS or whole cell. Its association with lipid and protein is controversial and is believed to be a chain which has not been incorporated into LPS. The rough strain *B. melitensis B*115 has been shown to synthesize smooth a chain which accumulates in the cytoplasm but is not assembled into complete LPS nor exported to the cell surface (Cloeckaert and Zygmunt, 1992).

The outer-membrane proteins include the outer-membrane of groups 1, 2, and 3, the most quantitatively important of which are the group 2 proteins. Other proteins associated with the peptidoglycan fraction of the outer membrane have been investigated as candidate protective antigen. In generat attempts to demonstrate protection with monoclonal or polyclonal antibodies to these have indicated low activity in the, absence of antibody to smooth LPS (Jacques and Cloeckaert., 1992). However, this does not disprove their role In protective immunity as they could still be implicated in relevant cell mediated responses (Corbel; 1998).

The A and M specific epitopes are actually present as minority structures in both Rand S (LPS). By means of slide agglutination, the A or M antigen predominance of *Brucella* strains can be determined (Wilson and Miles, 1932). This information is useful in classifying *Brucella* smooth nomen species into biogroups. Strains that are both A and M antigen

positive synthesize LPS with 0 chains that contain both A and M structural features in relatively high proportion (Perry and Bundle, 1990).

2.8. Susceptibility to physical and chemical agents:

The members of this group exhibit the usual susceptibility of vegetative bacteria to heat and disinfectants. In aqueous suspensions of moderate density they are destroyed by heating for about 10 min at 60°C but in very dense suspensions they may survive higher temperature. In agar cultures kept sealed at 4°C they generally survive for at least one month, and often longer. If lyophilized, especially in the presence of protecting agent, they will survive for decades (Corbel and Hendry, 1985).

Under natural conditions in favorable circumstances, *B. melitensis* may remain viable for 6 days in urine 6 weeks in dust and 10 weeks in water and soil. In pickled hams from naturally infected pigs, *B. suis* can live for as long as 3 weeks, but it is apparently destroyed. *B .suis* may live on sacking for 4 weeks and in sterile faeces for 100 days in the dark smoking (Corbel and Hendry, 1985). *B. abortus* may survive for 7 months in infected uterine exudates, if kept at about freezing point. It can also survive in bovine urine for 4 days, faeces 120 days, aborted fetuses for 75 days and liquid manure for up to 2.5 years. In raw milk at room temperature it seems to die out fairly rapidly with the production of acid. Acid production also seems to be the cause of its rapid death in butter, cheese and yoghurt although variable reports exist on its survival in these materials (Bang, 1897).

In general, survival in soft, non-acid cheese is much longe than in hard, lactic or propionic acid fermented cheese. Pasteurization, whether by holder or high-temperature short time cycles, kills brucellae in milk. Ultraviolet and ϒ-radiation at normal sterilization dosage are rapidly

lethal to *Brucella* provided that the organisms are not protected by the suspending medium. Ethanol, isopropanol, iodophors, phenols, hypochlorite, ethylene oxide, and formaldehyde (either gaseous or as aqueous formalin) are effective disinfectants for *Brulcella* under appropriate conditions; they are killed by 1.0% phenol in 15 min. Xylene and calcium cyanamide have been recommended for killing *Brucella* in manure but prolonged contact times are required (FAO/WHO, 2008).

2.9. Susceptibility to antimicrobial agents:

Good intracellular penetration is essential for in vivo activity against *Brucella* and thus there is limited correlation between in vitro performance and therapeutic efficacy. β-lactam antibiotics show limited activity against brucellae (Hall and Manion, 1970). Some strains are inhibited by benzylpenicillin, ampicillin and amoxycillin. Most strains are resistant to methicillin, nafcillin, ticarcillin and piperacillin. Similarly, first and second generation cephalosporins show limited activity against *Brucella* but some third generation cephalosporins such as cefotaxime, ceftizoxime, and cetfi-iaxone have minimum inhibition concentrations (MICs) in the range 0.25-2 mg P, (Palenque *et al*., 1986). Latamoxef is also active and has been used therapeutically, alone or in combination with rifampicin (Tosi and Nelson, 1982). The MIC for chloramphenicol is in the range 2-3 mg I^{-I} for most strains. Sensitivity to macrolides is variable. With the exception of *B. abortus* biogroup 2 and *B. ovis*, most strains are resistant to erythromycin, with $MIC_{90} \geq 16$ mg I^{-1}.

Sensitivity to dirithromycin and axithromycin show 2-8 fold greater activity (Garcia-Rodriuez and Munoz-Bellido, 1993). Most *Brucella* strains are inhibited by streptomycin, gentamicin, kanamycin, tobramycin and amikacin at concentrations of 1-4 mg I^{-I}, (Mortensen and Moore,

1986). Streptomycin augments the activity of tetracycline in infected cell cultures and this is borne out by therapeutic experience Sensitivity to tetracycline is universal, with MICs in regions of 0.1mg P (Hall and Manion, 1970).

Brucella strains are also highly sensitive to rifampicin; the MICs for rifampicin are in the range 0.1-2 mg I-I (Hall and Manion, 1970 and Corbel 1976). Single step resistance develops rapidly in vitro (Corbel 1976) and has also been observed during the course of therapy (Rautlin et nl., 1986). *Brucella* strains are generally resistant to nalidixic acid but show in vitro sensitivity to the floroquinolones. The MICs for ciprofloxacin are in the range O.5-lrng I^{-I} but therapeutic results have been disappointing (Bosch and Linares, 1986). Sensitivity to cotrimoxazole is borderline with MIC_{90} beingjust within the breakpoint. This is consistent with the high relapse rate observed with this drug (Garcia-Rodriuez and Munoz, 1993),

2.10. Growth requirements:

Most *Brucella* strains have much more exacting nutritional requirements, especially on primary isolation. In general several amino acids and minerals e.g. thiamin, biotin, nicotinamide, magneseium, Iron and manganese are required for growth. All *Brucella* strains are aerobic but some may grow best in a carbon dioxide-enriched atmosphere. No growth occurs in strictly anaerobic conditions. The optimum temperature for growth is about 37^0C but growth occurs in the range 20-40°C. For the isolation of *Brucella* from contaminated materials, the use of selective media is generally required (Adil., 2007).

Various formulations have been developed but the most generally

useful are based on that of Farrell (1974). This contains bacitracin, cycloheximide, nalidixic acid, nystatin, polymyxin Band vancomycin in a serum dextrose agar base. This formulation is too inhibitory for the isolation of some *Brucella* strains, particularly *B. avis*, for this, a medium containing vancomycin, colistimethate, nystatin and nitrofurantoin has been recommended (Brown *et aI.*, 1971). For the isolation of *Brucella* from human blood or bone-marrow samples, a 2-phase culture system of the type devised by Castaneda (1947) is recommended. However, Tryptone Soy and Thioglycolate broths are also used for blood culture, but contamination due to repeated subcultures is a possible consequent unlikely to occur with the Castaneda medium (Cheesbrough. 2000).

2.11. Epidemiology:

2.11.1 Epidemiology of brucellosis in humans:

2.11.1.1 Reservoirs of infection:

Brucellosis is a zoonotic disease, hence the ultimate sources of infection are infected animals. The key species are the major food-producing animals: cattle, sheep, goats, pigs. Others, including bison, buffalo, camels, dogs, horses, reindeer and yaks are less important, but they can be very significant local sources of infection in some regions. Recently, the infection has also been identified in marine mammals, including dolphins, porpoises and seals, and

these may present an emerging hazard to persons occupationally exposed to infected tissues from them. (Banai *et al., 2007)*

The risk of disease and its severity is to a significant extent determined by the type of *Brucella* to which an individual is exposed. This will be

influenced by the species of host animal acting as source of infection. (Ariza *et al.,* 2007)

B. melitensis is the type most frequently reported as a cause of human disease and the most frequently isolated from cases. It is the most virulent type and associated with severe acute disease. It is recorded as endemic in several countries and accounts for a disproportionate amount of human brucellosis. The organism is normally associated with infection in sheep and goats, but other species, including dogs, cattle and camels can be infected.(Banai *et al., 2007*)

In some countries, particularly in the Middle East, *B. melitensis* infection of cattle has emerged as an important problem. Contrary to some traditional views, *B. melitensis* remains fully virulent for man after infecting cattle. The bovine infection presents a particularly serious problem because of the large volume of infected milk that can be produced by an individual animal and because of the extensive environmental contamination that even single abortions or infected births can produce. (Ariza *et al.,* 2007)

Brucella abortus is the most widespread cause of infection, but associated with much less human disease. Infection in man is often sub-clinical and, where disease does occur, it is usually less severe than that caused by *B. melitensis* or *B. suis*. Cattle are by far the most common source of *B. abortus* but bison, buffalo, camels, dogs and yaks are important in some areas. (Ariza *et al.,* 2007).

Brucella suis has a much more restricted occurrence than *B. melitensis* and *B. abortus*. It is locally important as a source of human infection which can be as severe as that produced by *B. melitensis.* The sources and

virulence of the organism vary with its biovar (subtype defined by laboratory tests). (Ariza *et al.,* 2007)

Biovars 1, 2, and 3 are associated with pigs and also, in the case of biovar 2, with hares. This variant has a low pathogenicity for humans but biovars 1 and 3 are highly virulent and can cause severe disease. Biovar 4 is associated with infection of caribou and reindeer in Alaska, Canada and Northern Russia. (Banai *et al., 2007).*

It is infrequently reported as a cause of human disease. Naturally acquired human cases of biovar 5 infection have not been reported. (WHO; 2008).

B. canis is a widespread infection of dogs in many countries. It is infrequently associated with human disease. Reported cases have usually been mild. (WHO; 2008).

Brucella infection occurs in many species of wild animals but these are rarely implicated as sources of human disease. (Ariza J. *et al.,* 2007).

2.12. Transmission:

Transmission of *B. abortus* is very likely to occur via the oral route because cattle tend to lick aborted fetuses and the genital discharges of an aborting cow(Cunningham, 1977). Congenital infection can occur in newborn calves as a result of in utero infection and the infection may persist in a small proportion of calves which may also be serologically negative until after their first parturition or abortion (Blood *et al.*, 1983). Exposure to brucellae is also likely to occur when calves born to healthy dams and fed on colostrums or milk from infected dams Cunningham, 1977). It has been established that brucellosis in bulls does not always result in infertility, although semen quality may be affected. Bulls that remain fertile and functionally active will shed *Brucella* organisms with

the semen during the acute phase of the disease (McCaughey and Purcell.,1973).

Shedding, however, may cease or become intermittent (McCaughey and Purcell., 1973). In contrast to artificial insemination, bulls used in natural service may fail to spread the infection, as the infected semen is not deposited in the uterus (Ray, 1979). While indirect exposure to *Brucella* organisms could be mediated by wildlife, birds and waterways (contaminated with urine, uterine discharge of slurry from aborting cattle), it seems that only dogs carry pieces of placentae or aborted fetuses from one place to another causing direct exposure (Forbes., 1990).

Contamination of a cowshed or pasture takes place when infected cattle abort or have full-term parturition. Although it is generally accepted that *B. abortus* is =not excreted for any considerable time before abortion occurs, excretion in the vaginal discharges of infected cattle may occur as early as 39 days after exposure (Philippon *et aI.*, 1970). A massive excretion of brucellae starts after abortion and may continue for 15 days. Once the fetal membranes are expelled the uterine discharges diminish and the number of *Brucella* organisms excreted decreases rapidly (Nicoletti, 1980). Although the infectious material from the genital tract usually clears after 2-3 months, some infected cattle become carriers of Bnlcella and excrete it intermittently for many years (Philippon *et aI.*, 1970). Infected udders are clinically normal but they are important as a source of re-infection to uterus, to calves or human drinking their milk. (Ariza *et al.*, 2007)

Humans are generally infected in one of three ways: eating or drinking something that is contaminated with *Brucella*, breathing in the organism (inhalation), or having the bacteria enter the body through skin wounds

(Godfroid *et aI*., 2005). Consumption of contaminated foods (most likely eating or drinking contaminated milk or milk products) and occupational contact remain the major sources of infection for humans. Occupational disease is contracted by exposure of abattoir workers and veterinarians to infected(Ariza *et al.,* 2007).

Animals especially aborted fetuses, fluids, membranes or urine (Nimri., 2003). Inhalation of *Brucella* organisms is not a common route of infection/ but it can be a significant hazard for people in certain occupations/ such as those working in laboratories where the organism is cultured. Inhalation is also responsible for a significant percentage of cases in abattoir employees.

Contamination of skin wounds may be a problem for persons working in slaughterhouses or meat packing plants. Hunters may be infected through skin wounds or accidentally by ingesting the bacteria after cleaning deer, elk, mouse, or wild pigs that they have killed (Godfroid *et al*., 2005). Direct person-to-person spread of brucellosis is extremely rare. Mothers who are breast-feeding may transmit the infection to their infants. Sexual transmission has also been reported. Uncommon transmission may also occur VIa contaminated tissue transplantation (Geofrey *et al*., 2002).

2.13. Pathogenesis:

Although epidemiological evidence suggests that *B. abortus*, *B. melitensis* and *B. suis* show distinct host preferences, this only marks a general trend and the organisms are capable of establishing infection in a wide range of host species, including humans. *B. neotomae, B. canis* and *B. avis* in contrast, show much greater host specificity, and with the exception of occasional *B. canis* infections in carnivores and in humans

seem to have little capacity to spread beyond their usual hosts (Corbel, 1998).

2.13.1. Animal host:

Typically, in all host species *Brucella* grows intracellulary in the macraphages. Abortion is a frequent consequence of infection in the pregnant female, and orchitis and epididymitis can result in the male. Sexually immature animals are often less susceptible to. the disease. *Brucella* spp. has a predilection far the pregnant uterus, udder, testicle and the accessory male sex glands, lymph nades, jaint capsules and bursae. (Ariza *et al.*, 2007)

Erythritol, a substance produced by the fetus and capable af stimulating the growth of *Brucella* spp. occurs naturally in greatest concentration in the placental and fetal fluids and is probably responsible for localization of infection in these tissues. In the adult non-pregnant caw, localization occurs in the udder, and the uterus; if it became gravid, is infected from periodic bacteraemic phases originating in the udder. When the invasion af the gravid uterus occurs, the initial lesion is in the wall of the uterus and the spread to the lumen seen follows, leading to. a severe ulcerative endometritis of the inter-cotyledanary spaces. The allantachorion, fetal fluids and placental catyledons are next invaded and the villi destroyed (Blood, *et al*.,1983).

Abortion occurs principally in the last trimester of pregnancy, the incubation period being inversely proportional to the stage of development of the fetus at the time af infection (Blood *et aI*, 1983).

2.13.2. Human host:

The organism progress from the portal of entry, Via lymphatic channels and regional lymph nodes, to the thoracic duct and the blood stream, which distributes them to the parenchymatous organs. Granulomatous nodules may develop into abscesses from lymphatic tissues, liver, spleen, bone marrow, and other darts of the reticuloendothelial system. In such lesions, the Brucellae are principally intracellular. Osteomyelitis, meningitis, or cholecystitis also occasionally occur (Farrell., 1996). The main histological reactions in brucellosis consist of proliferation of mononuclear cells, exudation of fibrin, coagulation necrosis and fibrosis. The granulomas consist of epitheloid and giant cells, with central necrosis and peripheral fibrosis (Farrell., 1996).

The four *Brucellae* that infect humans have apparent differences in pathogenicity. *B. abortus* usually causes mild disease without supportive complications; non-caseating granulomas of the reticuloendothelial system are found. *B. canis* also causes mild disease. *B. suis* infection tends to be chronic with supportive lesions and caseating granulomas may be present. *B. melitensis* infection is more acute and severe. Persons with active brucellosis react more markedly (fever, myalgia) than normal persons to injected *Brucella* endotoxin. Sensitivity to endotoxin thus may play a role in pathogenesis (Farrell., 1996).

2.14. Diagnosis of brucellosis:

2.14.1. Human brucellosis:

The diagnosis of human Brucellosis is usually performed upon a set of clinical examinations and laboratory procedures. Symptoms (such as fever, headache, and loss of weight, profuse sweating and myalgia) and

physical signs (palpable spleen and liver, leucopenia, lymphocytosis) in addition to a clear history of exposure to brucellae is suggestive for " brucellosis. However, diagnosis should be confirmed by bacteriological and/ or serological means (Farrell., 1996).

2.14.1.1. Isolation of brucellae:

When infection is due to *B. melitensis* or *B. suis*, little difficulty is encountered in isolating the infecting organism from blood during the febrile episodes of the illness. But blood cultures are often negative in *B. abortus* infections. An attempt should always be made to isolate *Brucella* from patient1s blood during the febrile stage of the disease. Approximately, 5 ml of blood should be inoculated into blood culture bottles containing Tryptone soy broth. The bottles should be subcultured twice a week for 8 weeks on to Tryptone I soy agar. The Castaneda biphasic blood culture technique may be useful in reducing the risk of contamination during the long period of incubation. When *B. abortus* infection is a possibility, incubation should be in an atmosphere with added 5-10% C02. Blood cultures should be maintained for at least 8 weeks before they are discarded as negative (Adil 2007).

2.14.1.2. Serological tests:

When the infectious organism was not isolated from blood or other clinical materials, serological investigations of the patient is of paramount importance for the diagnosis of the disease and future management of the patient. As culture is not invariably successful, sera should be collected as soon as possible and at various stages of the illness. *Brucella* antibodies can be detected by a variety of serological tests. The most widely used are the Standard Agglutination Test (SAT),Rose bengal plate test (RBPT) and the Compliment Fixation Test (CFT) (WHO., 2008).

Additional information can be obtained by other techniques such as the Mercaptoethanol (ME) agglutination test, Radio-immunoassay (RIA), Enzyme-linked Immuno-sorbent Assay (ELISA) (WHO., 2008).

Recently, Polymerase Chain Reaction (PCR) has been used (AI-Attas *et al*, 2000). These techniques have been shown to be useful in the diagnosis of human Brucellosis.

As *Brucella* antibodies may be detectable for many years after acute or subclinical infection, the possibility of residual antibody from a previous infection must be borne in mind when considering the significance of *Brucella* antibodies in a patient's serum. False-positive reactions have been described due to cross-reactivity with strains of *E. coli, Salmonella urbana* and other group N serotypes, *Vibrio cholerae, Yersinia enterocolitica* and *Francisella tularensis* (Corbel., 1979). In persons whose symptoms are of recent onset, the presence of low titre of antibodies may be significant and in such instances a rising titre may be demonstrated by the SAT or CFT and can be of considerable help in confirming the clinical diagnosis. It is at this stage of the disease that the significant amount of Igt'v1 antibody is present; this is indicated by the presence of ME-sensitive agglutinins or can be shown more directly by ELISA or RIA (Farrell., 1996).

2.15. Treatment of brucellosis:

T**he essential** element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time. This should be within the context of general medical supervision and, for severely ill patients, is best carried out in hospital if circumstances permit. Antibiotic treatment should be implemented at as early a stage as possible, even in patients who appear to be showing a

spontaneous improvement. In those patients with complications, additional treatment, including in some cases surgical intervention, will be necessary. (WHO; 2008)

Uncomplicated acute brucellosis almost invariably responds well to appropriate antibiotic treatment. Patients and their families should be reassured that full clinical and bacteriological recovery is usual in human brucellosis. (WHO; 2008)

A variety of antimicrobial drugs have activity in vitro against *Brucella* species; however, the results of routine susceptibility tests do not always correlate with clinical efficacy. Consequently, beta-lactam antibiotics, such as penicillins and cephalosporins, and macrolide antibiotics, such as erythromycin, are associated with unacceptably high rates of relapse when used to treat patients with brucellosis. Although newer macrolides, such as azithromycin and

clarithromycin are more active in vitro than erythromycin, they have not shown superiority over current regimens for treatment of patients with brucellosis, and their role in therapy remains to be determined. (WHO; 2008)

2.16 Treatment of uncomplicated brucellosis in adults and children eight years of age and older:

2.16.1 Tetracyclines:

Tetracycline (500 mg every six hours orally) administered for at least six weeks has long been the standard treatment of human brucellosis. Doxycycline (a long acting tetracycline analogue) is now the preferred drug because it can be given once or twice daily, and is associated with fewer gastrointestinal side effects than tetracycline. Doxycycline is given

in a dose of 100 mg every 12 hours orally and is administered for a period of six weeks. (WHO; 2008)

2.16.2 Aminoglycosides

Because the rate of relapse when tetracycline or doxycycline are given alone remains between 10–20%, most authorities recommend an amino-glycoside to be given in addition to the tetracyclines for the first two to three weeks of therapy. (WHO; 2008)

Streptomycin (1 g/day intramuscularly) administered for two to three weeks has long been the aminoglycoside of choice when used in combination with tetracycline or doxycycline. Although synergy between the two drugs is difficult to prove using routine invitro assays, bacterial killing studies have shown that *Brucella* species undergo a more rapid rate of killing by the combination

than by either drug alone. (WHO; 2008)

Gentamicin is more active in vitro against *Brucella* species than streptomycin and, when administered as a single daily dose, is associated with few adverse side-effects. Although gentamicin, in a dose of 5mg/kg/day intravenously or intramuscularly, administered for 7 to 10 days in combination with doxycycline administered for six weeks, yielded good results in one study, experience with this regimen is too limited to justify its use over doxycycline plus streptomycin. (WHO; 2008)

Unfortunately, no direct study comparing the results of doxycycline plus streptomycin versus doxycycline plus gentamicin has yet been published. (WHO; 2008) .Until additional experience is gained using gentamicin in

place of streptomycin, the optimal dose and duration of therapy remain unknown. (WHO; 2008).

Key point on treatment of brucellosis in human :

• The essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time.

• Treatment of uncomplicated cases in adults and children eight years of age and older: doxycycline 100 mg twice a day for six weeks + streptomycin 1 g daily for two to three weeks.

OR

• Doxycycline 100 mg twice a day for six weeks + rifampicin 600–

900 mg daily for six weeks.

3. MATERIALS AND METHODS

3.1. Study area and duration:

This study was carried out in Khartoum State, including blood donors in Central Blood Bank, during: March – May 2014.

3.2. Study population and sample size:

One hundred and fifty (150) samples were collected randomly from blood donors,in Central Blood Bank,in Khartoum State.

3.3. Ethical consideration:

Approval to conduct this study was obtained from the College of Graduate Studies, Sudan University of Science and Technology.

Permission was obtained from the blood donors , in Central Blood Bank, in Khartoum State, samples were taken from donors after their consent.

3.4 Specimens Collection:

Aliquots of five mls of whole venous blood were collected using sterile disposable syringes. And left to colot to 5 to 10 minetes The collected specimens were transported to the laboratory, refrigerated overnight, centrifuged for 3000 r.p.m for 5 minetes and serum was separated and stored at -20°C until tested.

3.5. Laboratory Methods:

Two serological techniques were used to detect anti-brucella antibodies in collected sera. The Rose Bengal Plate Test (RBPT) was used as screening test and the Standard Agglutination Test (SAT) as a confirmatory test.

3.5.1. Rose Bengal Plate Test:

3.5.1.1. Principle:

The test depends primarily on the reaction between the *Brucella* antigen and the specific antibodies that was assumed to be present in the sera of examined subjects.

The standardized buffered Rose Bengal stained antigen was kindly provided by the Veterinary Research institute (VRI), at Soba and was used to screen all the obtained sera.

3.5.1.2. Procedure:

Rose Bengal Plate Test (RBPT) was performed according to (Cheesbrough., 2000). The serum samples and antigen were brought to room temperature. 30Microliter of each serum sample were placed on a white plastic plate. After shaking the antigen bottle, an equal volume of the antigen was placed near each serum spot. They were mixed thoroughly (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2 cm in diameter. The mixtures were agitated gently for 4 minutes at an ambient temperature on a rocker, after which the agglutination was read. Any visible agglutination was considered positive.

3.5.2. Standard Agglutination Test (SAT):

3.5.2.1. Principle:

This test was used to determine the antibody titer. It should also be performed when a patient with a negative test continues to show symptoms of brucellosis. The antigen reagent was kindly provided by VRI at Soba, Sudan.

3.5.2.2. Procedure:

Seven sterile small glass agglutination tubes labeled 1 to 7 were placed in a rack and 1 : 20 dilutions of serum was made in tube 1. One rnl from the first tube was taken and proceeded to make serial dilutions with 1 ml of 10% phenol saline up to the 6th tube, while the 7th tube was left to serve as a blank or negative control containing only phenol saline. Then one drop(50 microliter) of the antigen suspension (two antigen reagents (Omega-from Biosystem) one of the reagent was specific to *B. abortus* and the other was specific to *B. melitensis*) was added into each tube, mixed well and incubated at 37°C for 24 - 48 hours. Each sample was two folds serially diluted as follows: $\frac{1}{20}$, $\frac{1}{40}$, $\frac{1}{80}$, $\frac{1}{160}$, $\frac{1}{320}$, $\frac{1}{640}$ + control (normal saline + antigen reagents) was placed aside two reagents.

3.6. Quality control:

Positive and negative control sera were run in parallel with each performed batch. Duplicates of each tested serum were used to assure that the antigens used in the test were sensitive as well as specific (Cheesbrough., 2000).

3.7. Data analysis:

Data analysis was done, using the Statistical Package for Social Sciences (SPSS). Chi-square test was used to assess the difference between the various groups. Statistical significance was taken as $P(< 0.05)$.

4- RESULTS

Out of the blood samples collected from 150 donors , males were 133 (88.7%) , females were 17 (11.3%). Twenty three(15.3%) out samples of the 150 blood donors were positive for *Brucella* species.

Twenty (13.3%) samples were positive in males and 3(2.0%) in females donors.Table,1 and Fig,1.

As shown in table,2 student and employees had the higher incidenece of Brucellosis with percentages 43.5% and 39% respectively table,2

Table 3 revealed the prevalence of *Brucella* species found in the blood donors, 21 (70%) were *B.abortus* species and were 9(30%) *B.melitensis* table 3 and Fig 2

Seven samples showed antibodies of both *B.abortus* and *B.melitensis* all from males.

The distrubuation of *Brucella* species according to gender are showen table 4 and Fig 3. In males donors *B.abortus* were 20 (66.7%) and *B.melitensis* were 7 (23.3%) . in females donors 1 (3.3%) was *B.abortus* and 2 (6.7%) were *B.melitensis*

Table(1): The percentage of positive sample in male and female among the sex and total samples

Sex	Frequency	Positive	Percentage(total)	Percentage among sex
Male	133	20	13.3%	15%
female	17	3	2.0 %	17%
Total	150	23	15.3%	

Table(2): The percentage of ***Brucella*** isolates according to occupation of donors .

Occupation	No. of isolates	Percentage
Student	10	43.5%
Abattoir	2	8.7%
Employee	9	39%
Cooker	1	4.4%
Unemployed	1	4.4%

Table(3): The percentage of ***Brucella*** species among total brucella isolates

Brucella species	Positive	Percentage %
B. abortus	21	70%
B. melitensis	9	30%
Total of species	30	100%

Table(4): The percentage of ***Brucella*** species amonge male and female

Sex	species	***B. abortus***	***B.melitensis***
Male	27	20(66.7%)	7(23.3%)
Female	3	1(3.3%)	2(6.7%)
Total	30	21(70%)	9(30%)

Table (5) Titration Results for Brucellosis among blood donors

Code	1/20	1/40	1/80	1/160	1/320	1/640	Control
1		+(M)					
2			+(A)				
3				+(A)			
4			+(A)				
5				+(M)			
6			+(A)				
7				+(A)			
8			+(A)				
9				+(A)			
10			+(A)				
11				+(A)			
12				+(A)			
13			+(M)	+(A)			
14		+(M)	+(A)				
15		+(M)	+(A)				
16			+(A)				
17				+(A)			
18		+(M)		+(A)			
19			+(M)	+(A)			
20			+(M)		+(A)		
21			+(M)	+(A)			
22			+(A)				
23			+(A)				

A: *B*.abortus

M: *B*.melitensis

$\frac{1}{40}$ up to $\frac{1}{640}$ were considered positive for *Brucella* species.

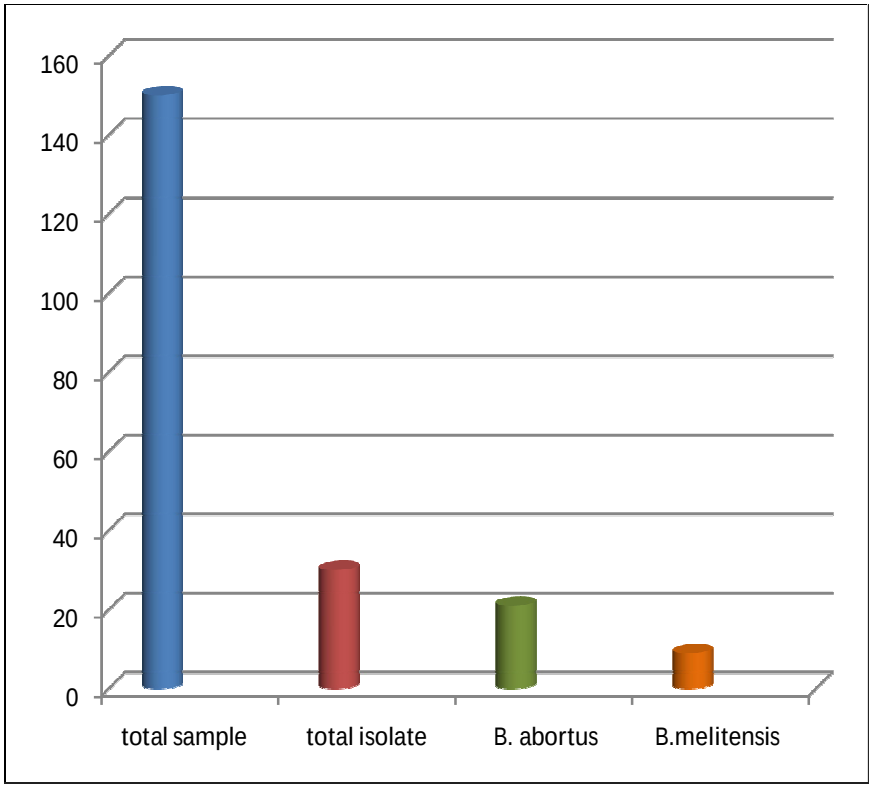

Fig 1: The percentage of ***Brucella*** species among total samples

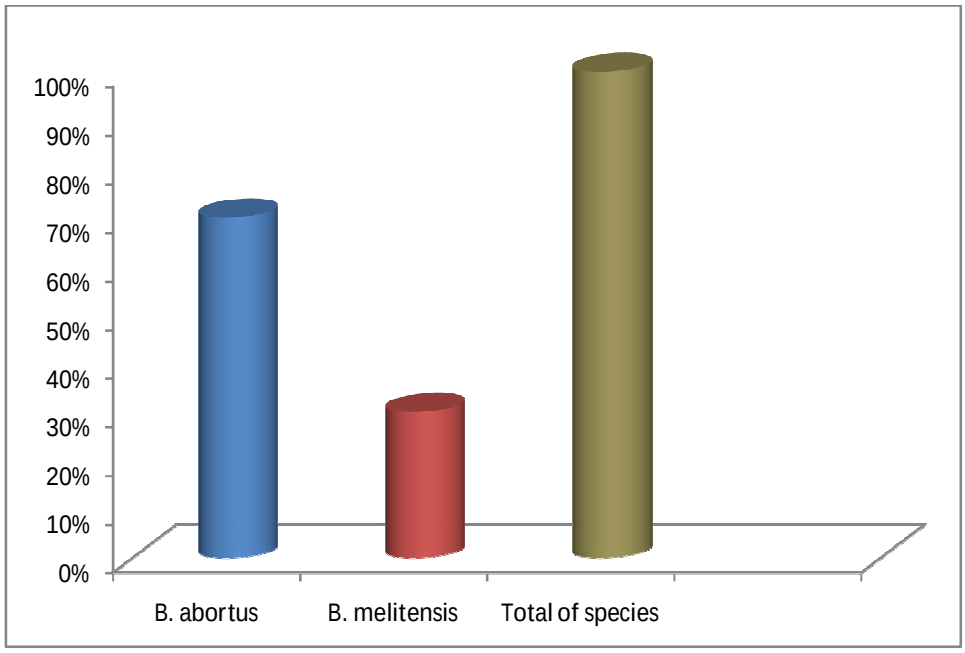

Fig 2: The percentage of ***Brucella*** species among the percentage of total ***Brucella*** isolates

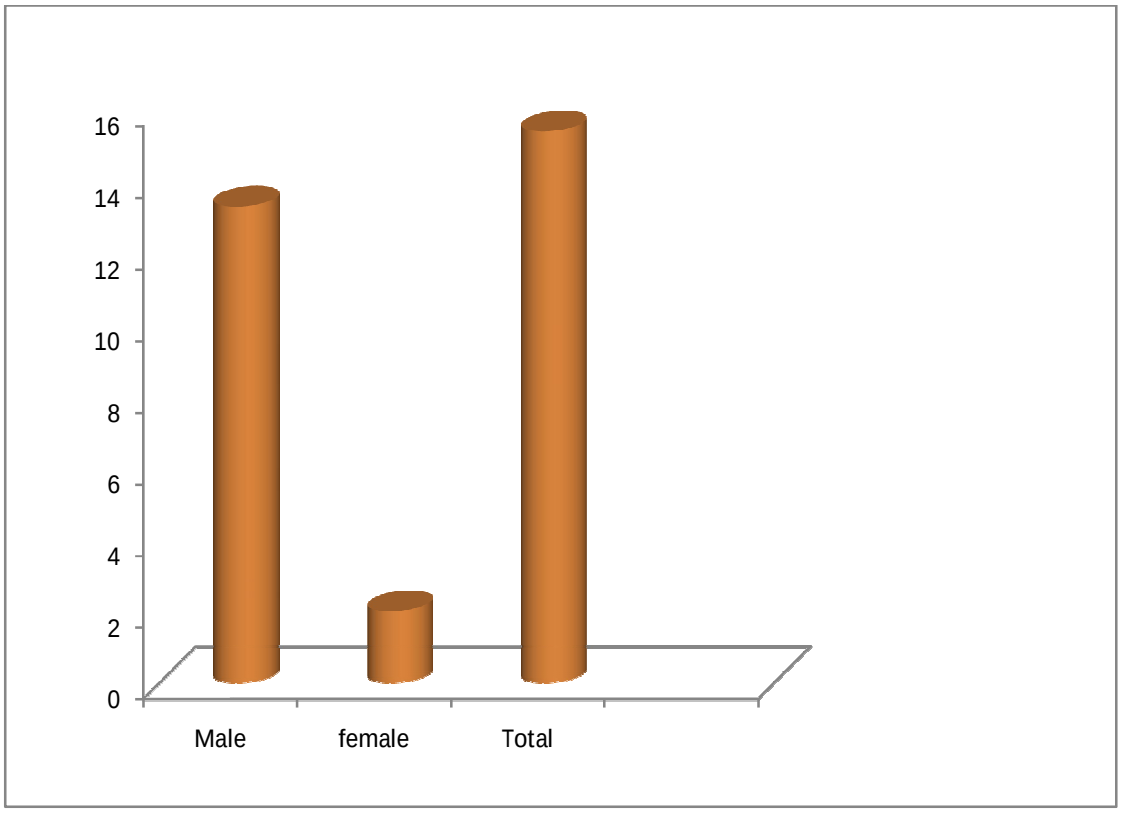

Fig 3: The percentage of positive sample in male and female among the percentage of total positive samples

5. DISCUSSION

5.1 Discussion:

Several studies were carried out on brucellosis in Sudan, but most of them were directed towards animal brucellosis Bennt.,(1943) , Dafalla and Khan ,(1958), Habiballa,*et al*., (1977), Sulima; (1987) , Musa *et al*., (1990) ,Suliman.,(2006). This study was attempt to understand the relation between blood donors and prevalence of brucellosis . The prevalence rate reported in this study was 15.3%, and it was higher than reported by (Mangalgi *et al., (2012)* in india and Bakhiet, (2004), Musa,(2004) who reported rate of 5.9% and 8% respectively in human in Sudan.

In my opinion, this difference might probably be due to the endemicity of *brucella* in Sudan . It is worthmenthioning that the occurrence of *brucella* in sudan is lower than the rate reported by Harding and Byers., (2000) . This high rate was attributed to the area of study where *brucella* is highly endemic.

students and employees had the higher incidenece of Brucellosis with percentages 43.5% and 39%. This may be due to bad hygeine mainly in type of food taken by these groups.

Out of the blood samples collected from 150 donors , males were 133 , females were 17 . Twenty (13.3%) samples were positive in males and 3(2.0%) in females donors. This may be due to the sample size .

In our opinion , there is risk of not including *Brucella* examination in the protocols of Sudanese blood banks. The risk is worsened by the fact that absence of symptoms even for along period does not necessarily ensure lack of infectivity and *Brucella* survive well in stored blood. Furtherly ,

this route of infection transmits brucellosis whereas most patients in need of blood transfusion are already weakened by severe disease. *Brucella* thus behaves very aggressively in such patients with higher risk of complication and fatalities .

This study showed significant probability value ($P<0.05$) among blood donors .

5.2 Conclusion

The high infection rate of *bruellosis* observed among the blood donors highlighted the risk of this pathogenic organism .

Prevalence of the disease among the blood donors are a potential and dangerous source to whom directly received the blood transfusion.

5.3 Recommendations

1. Screening of blood donors for *brucella* infection priors to donation.
2. Much knowledge about brucellosis has been accumulated, but not enough to master the disease. It is, therefore, necessary to acquire more knowledge.
3. To make this a reality, further in-depth studies are needed in human brucellosis by increasing the sample size of the people at risk and by employing the recent advanced molecular diagnostic techniques (e.g., PCR).
4. Because livestock animals and their products (meat, milk) are the major potential source for human infection, cooperation between veterinarians and human health care workers and epidemiologists is absolutely necessary to solve this problem once and for all.

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