INTRODUCTION

Brucellosis is a zoonosis caused by infection with the bacterial genus brucella, which are small aerobic intracellular coccobacilli. They are shed in large numbers in the animal's urine, milk, placental fluid and other fluids. Exposure to infected animals and their products causes brucellosis in humans **(Greenfield *et al.*, 2002)**.

It is a worldwide distributed disease, especially in Mediterranean countries and the Middle East and it remains a significant public health concern **(Mitka *et al.*, 2007**).

There are six species of brucellae, four of them are important for human. Br. Melitensis (infects sheep, goats and camels), Br. Abortus (infects cattle), Br. Canis (infects dogs), and Br. Suis (infects swine) **(Brooks *et al.*, 2007**).

Brucellosis in human can be presented as an acute infection, sub-acute or chronic infection. It may be presented as localized organ infection. In acute presentation, the patient complains of intermittent fever, rigors, profuse sweating, headache and arthalagia. If the diagnosis and treatment are delayed, localized organ involvement may occur such as musculoskeletal system, central nervous system, ocular, cardiac, respiratory, abdominal, genitourinary systems and hematological system **(Fauci *et al.*, 2008 and Nicki *et al.*, 2010)**.

The duration of human illness and its long convalescence mean that brucellosis is an important economic as well as medical problem

for the patients because of time lost from normal activities. Prompt diagnosis and treatment with antibiotics has greatly reduced the time a patient may be incapacitated **(Corbel, 2006)**.

The absolute diagnosis of brucellosis requires isolation of the bacterium from blood or tissue samples. The sensitivity of blood culture varies, depending on individual laboratory practices and how actively the obtaining of cultures is pursued. The percentage of cases with positive culture ranges from 15 to 70% **(Memish *et al.*, 2000)**.

There was no statistically significant difference between the results of ELIZA and agglutination tests **(Hussein *et al.*, 2010)**.

The goal of medical therapy in brucellosis is to control symptoms as quickly as possible to prevent complications and relapses. Multidrug antimicrobial regimens are the mainstay of therapy because of high relapse rates reported with mono-therapeutic approaches. The resistance is not a significant issue in treating brucellosis **(Maves *et al.*, 2011**).

According to age incidence, the age group 20-50 years formed 65.1% of brucellosis cases in a case control study conducted in Alexandria **(Meky *et al.*, 2007**). In relation to sex, the majority of brucellosis patients (64-70%) were males.

In Egypt two peaks were reported during the period from 1982 to 1991, one at 1986-1987 (51 cases reported for each year) and another higher peak at 1990-1991 (91 and 233 cases reported respectively) **(Wassif *et al.*, 1992)**.

Brucellosis is an important health problem in Egypt and a confirmed cause of 3% of cases of acute febrile illness **(Afifi S et al., 2005)**. However no more recent epidemiological studies are available.

Aim of the work

The aim of the present study is:

The aim of this work is to describe the epidemiological aspects of brucellosis in Egyptian patients in single center in terms of sociodemographic features, and risk factors.

Brucellosis

Definition:

Brucellosis is a zoonosis caused by bacteria of the genus brucella, which affects both humans and animals. Many names have been applied to it, often related to localities in which it was particularly prevalent; Malta fever, Mediterranean fever, Gibraltar or Rock fever, and undulant fever are probably the best known **(Park, 1997)**.

History:

Brucellosis and its etiologic agents are named after David Bruce, a Scottish physician who discovered the disease in 1887 while stationed in Malta. Dr. David Bruce researched Malta fever among British soldiers stationed on Malta in 1885. He described it as a disease clinically characterized by fever, profuse perspiration, splenomegaly, frequent relapses, rheumatoid or neuralgic pain, swelling of joint and orchitis **(Gotuzzo & Carrillo, 2003)**. Then, in 1887, he isolated the pathogen from the spleen of British soldiers who died as a consequence of this disease. Bruce named it Micrococcus melitensis, derived from Melita (honey), the Roman name of the Isle of Malta **(Bruce, 1887, Godfroid *et al.*, 2005 and Mantur *et al.*, 2007)**.

Hughes, a colleague of Dr. Bruce, in a monograph in 1897, confirmed the Bruce finding and discussed clinical and pathological finding in 844 patients in more details and suggested the name of "undulant fever" **(Rust *et al.*, 2006 and Purcell *et al.*, 2007)**.

Hughes' term "undulant fever" became the most widely accepted clinical designation until "brucellosis" obtained currency, although "Malta fever" has also shown some staying power as a designation **(Rust *et al.*, 2006)**.

In 1905, Dr Zammit and other Maltese doctors working in this commission established the zoonotic principle of the role of animals in transmission of the disease to human. They demonstrated that more than half of Maltese goats were asymptomatically infected and that the organism could be transmitted to humans by the consumption of raw milk and milk products or by contact with infected goat urine **(Gotuzzo & Carrillo, 2003 and Rust *et al.*, 2006)**.

In cattle, the disease firstly appears as epidemic abortion in Latin America in 1864 and regarded as contagious abortion **(Hall, 1989)**. In 1897, Bernard Bang, a Danish veterinarian from Copenhagen, identified an intracellular microorganism described as 'Bacillus abortus' as the cause of abortion in cattle. The disease was named for him, 'Bang's Disease' in 1930 **(Nicoletti, 2002 and Mantur *et al.*, 2007)**.

In 1918, Alice Evans an American bacteriologist proved that Micrococcus melitensis from goats and Bacillus abortus from cows could not be differentiated morphologically or by cultural and biochemical reactions but there were an antigenic differences which could be shown by agglutination absorption test. In the same year she

suggested the possible pathogenicity of Bacillus abortus to man **(Evans, 1918 and Mantur *et al.*, 2007)**.

In 1920, Evans confirmed that Micrococcus melitensis was a bacillus and not, as originally described a micrococcus. She also showed that Micrococcus melitensis, Bacillus abortus and isolates from pigs belonged to one genus **(Hall, 1989 and Mantur *et al.*, 2007)**.

Meyer and Shaw further confirmed Evans` observations and suggested the generic name brucella in the honor of Dr. David Bruce **(Mantur *et al.*, 2007)**.

Microbiology of brucellosis

A- Organism:

Brucella have been traditionally classified according to its differences in pathogenicity and preferred hosts into six named species: Brucella melitensis principally within sheep and goats, Br. Abortus in cows, Br. Suis in pigs, Br. Canis in dogs, Br. Ovis in sheep and Br. neotomae in rodents **(Mantur *et al.*, 2007 and CFSPH, 2009)**.

Organisms of the genus brucella appear as cocci, coccobacilli and short bacilli, 0.5-0.7µ wide by 0.6-1.5µ long. They occur singly, in groups or short chains. They are Gram-negative, non motile, non capsulated and non sporing **(Corbel, 2003)**.

B- Characteristics and growth:

Brucellae can grow slowly in vitro on enriched media, such as serum dextrose agar, optimally at 37Cº. B.abortus and Br.Ovis grow better in the presence of added CO2; slow growth in vitro requires cultures to be maintained and subclutured blindly for up to 6 weeks before discharging clinical samples for culture **(Wright, 2003)**.

Brucella is facultative intracellular bacteria that cause brucellosis in variety of animals and undulant fever in humans **(Boschiroli *et al.*, 2001)**.

Brucella is Gram negative bacteria (bacilli) it is intracellular bacteria especially in the reticuloendothelial system so it has the ability for chronicity and relapse **(Nicki, 2010)**.

The ability of these organisms to survive in professional and non professional phagocytic cells is the basis for disease **(Ficht, 2003)**.

C- Reservoir:

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 buffalo, camels, dogs and horses. 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. 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 **(WHO, 2006 and CFSPH, 2009)**.

Br. 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 **(WHO, 2006)**.

Also, infection of equine with Br. Abortus occurs after contact with infected cattle or other maintenance hosts **(Acha & Szyfres 2003 and AVMA, 2007)**.

Br. Canis is a widespread infection of dogs in many countries. It is infrequently associated with human disease. Reported cases have usually been mild **(WHO, 2006)**.

D- Transmission of brucellosis to humans:

D-1-Foodborne transmission:

This is usually the main source of brucellosis for urban populations. Ingestion of fresh milk or dairy products prepared from unheated milk is the main source of infection for most populations. Cow, sheep, goat or camel milk contaminated with Br. Melitensis is particularly hazardous as it is drunk in fairly large volume and may contain large numbers of organisms. Butter, cream or ice-cream prepared from such milk also presents a high risk. Soft cheeses prepared from sheep or goats milk by addition of rennet are a particularly common source of infection in Mediterranean and Middle Eastern countries. The cheese-making process may actually concentrate the brucella organisms, which can survive for up to several months in this type of product. Such cheeses should be stored in cool conditions for at least six months before consumption. Hard cheeses prepared by lactic and propionic fermentation presents a much smaller risk. Similarly, yoghurt and sour milk are less hazardous. Brucella dies off fairly rapidly when the acidity drops below pH4, and very rapidly

below pH3.5. Equipment used in the transport or processing of infected milk or other raw material may contaminate uninfected products unless good hygienic practice is observed **(WHO, 2006)**.

Meat products are less frequently associated with infection, mainly because they are not usually eaten raw. Another cause, that muscle tissue usually contains low concentrations of brucella organisms but liver, kidney, spleen, udder and testis may contain much higher concentrations. In some countries, dishes prepared from these organs may be eaten raw or undercooked so All meat should be cooked until it reaches an internal temperature of 145 to 165 F (63 to 74 C) **(Mantur & Amarnath, 2008)**.

D-2- Occupational exposure:

Also, humans can contract brucellosis through direct contact with infected animals and their products such as; aborted fetuses, placentas, vaginal discharges, blood, feces and urine. The organism enters the body by inoculation through abrasions of skin and mucous membranes **(Bossi *et al*., 2004and Pappas *et al., 2005)***.

Brucellosis is regarded as an occupational disease for persons who work with farm animals, especially cattle, sheep, goats and pigs such as farmers, farm laborers, animal breeders, veterinarians and inseminators. As well as, persons involved in the processing of animal products may be at high risk of exposure to brucellosis, especially slaughter men, butchers, meat packers and cheese factory workers **(WHO, 2006)**.

Patients those had the history of exposure to both risk factors (dietary and animal contact) formed (62.3%) of patients **(Mantur *et al.*, 2004)**.

D-3- Inhalation of infectious aerosols:

Inhalation of brucella organisms is not a common route of infection, but it can be significantly hazardous for people in certain occupations, such as those working in laboratories where the organism is cultured and in abattoir employees **(Baron, 2005)**.

Laboratory acquired Brucella infection is a major health hazard for the laboratory workers handling the cultures of the virulent or attenuated strains as 2% of all brucellosis cases in industrialized countries are laboratory acquired **(Yagupsky & Baron, 2005 and CDC, 2008)**. The incidence rate in cases of accidental laboratory exposure ranges from 30 to 100%, depending on the location of workers and the quantity of bacteria involved **(Fiori *et al.*, 2000)**.

D-4- Infection from a contaminated environment:

This is difficult to document but probably occurs more frequently than is recognized. Infected animals passing through populated areas or kept in close proximity to housing may produce heavy contamination of streets, yards and market places, especially if abortions occur. Inhalation brucellosis may then result from exposure to contaminated dust, dried dung etc. Infection may also result from contamination of skin or conjunctivae from soiled surfaces. Water sources, such as wells,

may also be contaminated by recently aborted animals or by run-off of rain water from contaminated areas **(WHO, 2006)**.

D-5- Travel-acquired brucellosis:

Tourists or business travelers to endemic areas may acquire brucellosis, usually by consumption of unpasteurized milk or other dairy products. Travelers may also import infected cheeses or other dairy products into their own countries and infect their families or social contacts by this means. Imported cases now account for most of the acute brucellosis cases seen in North America and Northern Europe **(Al Dahouk *et al.*, 2005)**.

D-6- Person-to-person transmission:

This is extremely rare. Occasional cases have been reported in which circumstantial evidence suggests close personal or sexual contact as the route of transmission **(WHO, 2006)**.

E- Pathogenesis:

Brucella are facultative intracellular organisms of the reticuloendothelial system. Animal studies suggest that invading brucellae are rapidly phagocytozed by polymorphonuclear leukocytes **(Alton & Forsyth, 1996)**.

The brucella may enter the body through digestive tract, lungs or mucosal layers and intact skin **(Lapaque *et al.*, 2005)**.

Then polymorphonuclear cells and activated macrophages migrate to the site of entry of brucella organisms. During the early phase of invasion, extracellular killing is carried out by IgM and

complement mediated mechanisms. However, brucella organisms can resist such killing **(Madkour, 2003)**. The organism is able to escape phagocytic killing through inhibiting the phagosome-lysosome fusion and reproducing inside macrophages. The interaction between the organisms and macrophages will determine the severity and outcome of infection **(Young, 2005)**.

During the early stage of the disease, patients are frequently bacteraemic that has a continuous pattern, making circulating Brucella easily detectable by blood culture. Once in the blood stream, the organism is seeded to multiple organs/systems, especially those rich in reticuloendothelial tissue, such as liver, spleen, skeletal and hematopoietic system **(Greenfield *et al.*, 2002)**.

The cytotoxic activity of natural killer cells, with decrease in the CD4+ and increase the CD8+ lymphocyte subpopulations is depressed in patients with active brucellosis .Cytokines including interleukin 12 and tumor necrosis factor appear to play an important role in host defense against brucella infection **(Madkour, 2003)**.

The pathogenicity of brucella to humans is varied according to its species; Br. Melitensis have the highest pathogenicity; Br. Suis have high pathogenicity; Br. Abortus and Br. Canis have moderate pathogenicity **(Maloney, 2008)**.

F- Bio-terrorism:

Br. Melitensis and Br.Suis have been developed experimentally as biological weapons by state sponsored programes. Their relative stability in aerosol forms, combined with low infectious dose make them suitable agents for this purpose. Brucella could be used to attack human and/or animal populations. The impact is likely to be greatest in those areas in which the disease is not endemic. The organism can be obtained from natural sources in many parts of the world. Health and veterinary authorities should be aware of this potential source of infection **(Corbel, 2006)**.