Ministry of higher education and scientific research

University of Diyala

College of medicine

Brucellosis

A project submitted to the council of College of Medicine / University of Diyala in Partial fulfillment of the Requirements for the Degree of bachelor in medicine and General surgery

Presented by :

Hamza Ahmed Mohammed

Supervised by :

Professor Dr. Abdulrazak SH. Hassan

((Medical Microbiologist))

٢٠٢١

الاهداء

الاهداء اولا الى وجه الله تعالى

في جميع مراحل الحياة يوجد اناس يستحقون منا الشكر والتقدير واولى الناس بالشكر

والدي المرحوم الذي جرع الكاس فارغا ليسقيني قطرة حب

والدتي المرحومة التي وضعتني على طريق الحياة وكان لها فضل كبير في نجاحي

والى جميع من وقفوا بجانبي وساعدوني

الى جميع اساتذتي الكرام بوجه عام والى الى **الاستاذ الدكتور عبد الرزاق شفيق حسن**

المشرف على البحث بوجه خاص والذي كان له دور كبير في اعطائي المعلومات القيمة

اهدي لكم بحث تخرجي واتمنى ان يحوز رضاكم

الشكر والتقدير

قال رسول الله (صلى الله عليه وسلم)

((من لم يشكر المخلوق لم يشكر الخالق))

الحمدلله الذي هدانا واعدنا وامدنا والهمنا الصبر على المشاق ووفقنا لما نحن عليه

فله الحمد والشكر

ارفع كلمة الشكر والتقدير الى **الاستاذ الدكتور عبدالرزاق شفيق حسن**

وفقه الله فقد كان عونا وسندا لي

والى كل من مد يد العون لي من قريب او بعيد

وقبل ان امضي اقدم اسمى ايات الشكر والامتنان والمحبة والتقدير الى الذين مهدوا لي طريق العلم والمعرفة

Abstract

Brucellosis, also called Malta fever, is a bacterial infection caused by Brucella species. It is a zoonotic disease that infect human mainly by the infected byproduct especially cow milk. It's a worldwide problem, because it's potential harm effect on livestock and humans and considered the leading neglected zoonotic disease. Its clinical manifestations are summarized by fever, malaise, headache and constipation. Treatment with antibiotics is available and can eradicate the infection completely in few days. No vaccine is available and vaccinating the vulnerable animal is the best preventive measure.

Introduction

Brucellosis, also known as "undulant fever", "Mediterranean fever" or "Malta fever", is a zoonosis that is almost always transmitted by contact with infected animals or their products. It has an effect on people of all ages and genders (Corbel, 2006). An infectious disease is wreaking havoc on livestock productivity and human health all over the world. Because of the disease's disproportionate impact on low-income countries, the World Health Organization (WHO) has designated it as one of the world's most "neglected zoonotic diseases" (franc et al., 2018). While several countries have made significant strides in controlling the disease, there are still areas where the infection persists in domestic animals and, as a result, transmission to humans is common (Corbel, 2006).

Since the time of Hippocrates in 450 B.C., it has been known along the Mediterranean littoral. J. A. Martson, a British Medical Department assistant surgeon employed in the Mediterranean in 1861, was the first to identify the symptoms of brucellosis in himself as gastric remittent fever (Manish et al., 2013). Professor L. F. Benhard Bang, a veterinary pathologist and bacteriologist from Denmark, worked with Stribolt to isolate the small bacillus that is now known as *Brucella abortus*. In 1897, a US army officer was diagnosed with human brucellosis for the first time (Brown, 1977). Captain David Bruce, a Scottish surgeon, discovered small coccal

organisms in stained portions of a fatally infected soldier's spleen and isolated and named the organism in culture from spleen tissue of four other British soldiers stationed in Malta, Melita (honey), the Roman name for the island of Malta, inspired the organism's name (Manish *et al*., 2013).

Human brucellosis was first confirmed in Iraq in 1938 when the microorganism was first isolated by an Iraqi clinician. Its relatively common infectious disease especially in Iraqi Kurdistan, it is a widely spread disease and remains a health problem there (Al bayaa, 2017). In this short article, we will focus on the epidemiology, bacteriology, pathogenesis, mode of transmission, the clinical features and the incidence in Iraq.

Microbiology

The genus *Brucella* belongs to the alfa-2 subgroup of the class proteobacteria. Six main species are distinguished: *B. abortus, B. suis, B. melitensis, B. neotomae, B. ovis, B. canis* (Corbel, 2006). Not all of them are pathological to human, hence *B. neotomae and B. ovis* cannot cause human brucellosis. *Brucellae* are small, non-motile, non-sporulating, nontoxigenic, non-fermenting, facultative, intracellular, gram-negative coccobacilli. Flagella, endospores and capsules are absent although capsule like structures have been reported n preparations treated with antiserum. They are partially acid-fast, as are not decolorized by 0.5% acetic acid in the modified Ziehl-Neelsen (MZN) staining (Alton, 1988).

The genome of *Brucella* has been sequenced and is made up of two chromosomes. Important genes encoding for metabolic and replicative functions can be found on both chromosomes. In Brucella, no naturally occurring plasmids have been discovered. Brucella, on the other hand, may keep some plasmids that have been introduced through electroporation or conjugative transfer (DelVecchio *et al.*, 2002).

Transmission

Direct contact with the placenta, fetus, fetal fluids, and vaginal discharges or byproducts (e.g., milk, meat, and cheese) from infected animals is the most common way for *Brucella* species to infect humans (Liu et al., 2018). This explains why direct

ingestion, mucous membranes, broken skin, and in rare cases intact skin are the most common routes of infection. In addition, transmission in utero, transmission from person to person, and transmission associated with tissue transplant have all been observed in rare cases (Tuon *et al.*, 2017).

Epidemiology

Brucellosis is a disease that is constantly evolving or re-emerging around the world, and the epidemiology of the disease has changed significantly in the last decade. Human brucellosis cases are thought to be up to 26 times higher than the 500,000 cases reported each year. Significant endemic areas include the Mediterranean basin, the Middle East, including Iraq and Iraqi Kurdistan, the Indian subcontinent, and parts of Mexico, Central and South America (Bosilkovski *et al.*, 2007).

Brucellosis is becoming more prevalent in many developing countries, despite advances in surveillance and control, due to a variety of sanitary, socioeconomic, and political factors. In Iraqi Kurdistan, human brucellosis still has a high prevalence and incidence, with cases recorded in all three provinces. In 2012, the prevalence rate in Erbil city was 10.7%, and in Dohuk, it was 6.36 percent. Sulaimani province, which has the highest brucellosis incidence in Kurdistan and Iraq, recorded 976 cases of the disease in 2013 (Mohammed, 2015).

Pathogenesis

Both phagocytic and non-phagocytic cells help and multiply Brucella strains. Macrophages, dendritic cells, and trophoblast cells are the bacteria's key targets. *Brucella* may, however, replicate inside other cells, such as epithelioid cells (Celli, 2006). *Brucella* spreads through the mucosal epithelial cells layer, where it is engulfed by competent phagocytes (macrophages and DC cells). *Brucella* lives for up to 72 hours in non-phagocytic cells before breaking through the epithelial barrier and infecting phagocytic cells (Głowacka, 2018).

Penetration into the epithelial cell requires actin polymerization. The adhesion of *B. abortus* to the cell surface leads to activation of GTPases of Rho subfamily, *e.g.*

Rho, Rac, and Cdc42 (Kim, 2015). These proteins are involved in cytoskeletal regulation and have an impact on parasitic bacterial internalization. Cdc42 is the only GTPase activated directly by *B. abortus* during the contact with a no phagocytic cell. It seems that other GTP-ases (Rho or Rac) are activated indirectly, because their inhibition impede invasion into host cells (Głowacka, 2018).

After a few minutes of invasion, the bacteria begin to interact with the host cell's usual structural proteins. Both sensitive and nonspecific phagocytes are thought to have identical sequallae. The bacterium enters the cell and begins to form BCV (Brucella containing vacuoles), which interact with the cytoplasmic endosomic receptors. After 10 minutes, the cell begins to react to the BCV by acidifying the vacuoles, which is a critical step in bacterial survival (Głowacka, 2018).

Clinical presentation

Depending on the virulence of the organism, the route of entry, and the infectious dosage, the incubation duration can vary. Acute (less than eight weeks), subacute (from eight to 52 weeks), and chronic (more than 52 weeks) brucellosis cases are randomly categorized based on the length of symptoms (Mandell, 2010). Brucellosis has a wide clinical scope, ranging from a moderate febrile illness to serious multisystem involvement. Fever is the most common clinical symptom; fifty percent of people experience a sudden onset of fever in the evening. Patients that have been untreated for a long time have erratic fever patterns with rising temperatures and chills. (Ulu, 2013).

Acute brucellosis is marked by a recurrent or intermittent fever. Other signs of acute brucellosis include malaise, fever, weight loss, arthralgia, myalgia, constipation, anorexia, and backache. With *Brucella melitensis*, acute presentation is more typical than with other species. In endemic areas, subacute brucellosis is the most common and classical type, with undulant fever. Fatigue, headache, and myalgia are the most common symptoms. Furthermore, localized infections such as epididymitis, orchitic and osteoarticular complications are more commonly seen.

Chronic brucellosis usually due to persistence of the infectious foci in the kidneys or bone and charcterized by fever , fatigue and depression (Doganay,2003).

Diagnosis

The medical history, physical examination, and sufficient laboratory tests, as well as their understanding, are all essential for brucellosis diagnosis. Blood and other tissue cultures (vs. bone marrow) are the gold standards for diagnosis; however, bacteria cannot always be isolated. The organism's isolation is often ineffective, particularly in chronic cases, or takes a long time. As a result, serological tests are being used more often (Araj, 2010). Rose Bengale test (RBT) is a rapid slide agglutination test which depends on the reaction of serum and suspension of *B. abortus*. RBT is widely used as a screening test with a high sensitivity. However, false negative results have been reported with RBT, especially in chronic cases (Araj, 2010).WHO also recommends confirming RBT positive results by the SAT, due to reduced specificity in endemic regions.

Serum agglutination test (SAT) detects antibodies against smooth lipopolysaccharide (s-LPS), the major antigen of the bacteria. These antibodies persist after recovery, affecting diagnostic value of the test. STA test is more sensitive in the diagnosis of acute brucellosis than chronic cases (Franco, 2007). To rule out the effect of blocking or incomplete antibodies, the anti-human globulin test (Coombs) is needed. This test is particularly useful in cases that are complicated or chronic. ELISA is a standardized, quick (4-6 hours), and sensitive method for detecting antibodies to s-LPS. The test determines how many agglutinating antibodies there are in total and on an individual basis (IgG, IgM and IgA). IgG subclasses and IgE are also detected by this examination. When other tests come back negative, ELISA is highly recommended (Zhang, 2010). A sensitive diagnostic tool, PCR, is also available.

Treatment

The standard treatment for human brucellosis has long been tetracycline (500 mg orally every six hours) given for at least six weeks. Doxycycline (a long-acting tetracycline analogue) has replaced tetracycline as the favored antibiotic since it can be used once or twice daily and has less gastrointestinal side effects. Doxycycline is taken orally in doses of 100 mg per 12 hours for six weeks (Corbel, 2006).

Since the rate of relapse with tetracycline or doxycycline alone is 10–20 percent, most authorities suggest using an aminoglycoside in addition to the tetracyclines within the first two or three weeks of treatment. Streptomycin (1 g/day intramuscularly) has long been the aminoglycoside of choice when combined with tetracycline or doxycycline for two to three weeks. Rifampicin is effective against Brucella species in vitro, has a high lipid solubility, and accumulates in eukaryotic cells (Corbel, 2006).

Prophylaxis

Currently, there is no vaccine available to prevent the infection. The only prophylactic measure is to vaccinate the livestock and the susceptible hosts in the endemic area. Caring for both occupational and personal hygiene, continuous sanitation and disinfecting the farms and wearing protective gloves when dealing with animal, considered effective protective measures.

Complications

Osteoarticular system

Osteoarticular involvement is the most common focal complication of brucellosis. The incidence of osteoarticular involvement is 10-85% in most series. Sacroiliac joints are the most common site involved in younger patients, whereas spondylitis and peripheral arthritis usually occur in older patients (Turan, 2011).

Gastrointestinal system

After entry into the body, bacteria localize in the tissues of the reticulo-endothelial system. The liver is frequently affected as being the largest organ of the reticulo-endothelial system. Hepatic enlargement is the most frequent gastrointestinal

manifestation of brucellosis, has been reported 32- 63% of cases. Hepatitis, granuloma and/or abscess formation due to brucellosis are rare conditions (Madkour, 2003).

Respiratory System

Involvement of the respiratory system in brucellosis is rare (< 5%), however, a variety of pulmonary manifestations were reported, including acute bronchitis, bronchopneumonia, pleural effusion, empyema and lung abscess. Most frequently reported symptoms are dry or productive cough dyspne, chest pain and flu like symptoms with an incidence of 15-30% (Doganay, 2003).

Nervous system

Neurobrucellosis is a rare but serious complication of brucellosis. Headache , weight loss, back pain and dizziness and rarely pseudotumour cerebri have been reported in patients with brucellosis. Some psychiatric symptoms like depression and motion liability also occur (Ulu, 2013).

Cardiovascular system

cardiovascular complications of brucellosis (endocarditis, myocarditis and pericarditis) are rare (1% of cases).

Conclusion

This reviewing dissertation can concluded that, in Iraq, Brucellosis is among neglected diseases in spite of its high prevalence all over the country. Actually, the situation is highly complicated by the high prevalence of the disease among domesticated animals and animal products. The intracellular nature of the brucella species make it as a latent pathogen that able to sequestrated itself away from the reach of medication and flare up thereafter. For this reason and by the time, the brucella become extensively resistant to most available antibiotics. So, complete cure or elimination of the microbe from the body rarely occur. The ideal control measure is by vaccination of the community. Till that time the disease will continued as an out of control.

References

1. Al-Bayaa YJ. Epidemiology of Human Brucellosis among Populations in Iraq's Provinces in 2015. Journal of the Faculty of Medicine Baghdad. 2017 Jul 2;59(2):165-9.
2. Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the brucellosis laboratory. Institut National de la recherche Agronomique (INRA); 1988.
3. Araj GF. Update on laboratory diagnosis of human brucellosis. IntJ Antimicrob Agents. 2010; 36: 12-7
4. Bosilkovski M, Dimzova M, Grozdanovski K. Natural history of brucellosis in an endemic region in different time periods. Acta clinica croatica. 2009 Mar 2;48(1):41-6.
5. Brown GM, GM B. The history of the brucellosis eradication program in the United States;1997.
6. Celli J. Surviving inside a macrophage: the many ways of Brucella. Research in microbiology. 2006 Mar 1;157(2):93-8.
7. Corbel MJ. Brucellosis in humans and animals. World Health Organization; 2006.
8. DelVecchio VG, Kapatral V, Elzer P, Patra G, Mujer CV. The genome of Brucella melitensis. Veterinary microbiology. 2002 Dec 20;90(1-4):587-92.
9. Doganay M, Aygen B. Human brucellosis: an overview. International journal of infectious diseases. 2003 Sep 1;7(3):173-82.
10. Franc KA, Krecek RC, H?sler BN, Arenas-Gamboa AM. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. BMC public health. 2018 Dec;18(1):1-9.
11. Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. The Lancet infectious diseases. 2007 Dec 1;7(12):775-86.
12. Głowacka P, Żakowska D, Naylor K, Niemcewicz M, Bielawska-Drozd A. Brucella–virulence factors, pathogenesis and treatment. Polish journal of microbiology. 2018 Jun;67(2):151.

13. Liu F, Li JM, Zeng FL, Zong Y, Leng X, Shi K, Diao NC, Li D, Li BY, Zhao Q, Du R. Prevalence and risk factors of brucellosis, Chlamydiosis, and bluetongue among sika deer in Jilin Province in China. Vector-Borne and Zoonotic Diseases. 2018 Apr 1;18(4):226-30.
14. Madkour MM. Madkour's brucellosis. 2nd ed. New York, NY: Springer-Verlag; 2001
15. Mandell GL. Douglas, and Bennett's principles and practice of infectious diseases. Philadelphia, PA: Churchill Livingstone.
16. Manish K, Chand P, Rajesh C, Teena R, Sunil K. Brucellosis: an updated review of the disease. Indian Journal of Animal Sciences. 2013 Jan 1;83(1):3-16.
17. Mohammed IS. Environmental, efface and filed study for source Brucella disease. International Journal of Advanced Research. 2015;3(7):177-84.
18. Tuon FF, Gondolfo RB, Cerchiari N. Human-to-human transmission of Brucella–a systematic review. Tropical Medicine & International Health. 2017 May;22(5):539-46.
19. Turan H, Serefhanoglu K, Karadeli E, Togan T, Arslan H. Osteoarticular involvement among 202 brucellosis cases identified in Central Anatolia region of Turkey. Internal Medicine. 2011;50(5):421-8.
20. Ulu Kilic A, Metan G, Alp E. Clinical presentations and diagnosis of brucellosis. Recent patents on anti-infective drug discovery. 2013 Apr 1;8(1):34-41.
21. Zhang X. Brucellosis indirect enzyme-linked immunosorbent assay antibody detection kit. CN101799470. 2010.