

Histopathological changes in liver and spleen of mice infected with brucellosis

Sameeah M. IHamad* ,Abeer YousifAbd Al-Kareem* NajimAldin-Abdulla**



*University of Anbar-Collage of Medicine.

** University of Anbar-Faculty of Dentistry,

ARTICLE INFO

Received: 19 / 5 /2022
Accepted: 28 / 5 /2022
Available online: 19/7/2022
DOI: 10.37652/juaps.2014.123908

Keywords:

Brucella,
liver,
spleen.

ABSTRACT

A total of 20 female mice (8 week of age)a 10 mice control and 10 deal with 10⁵cfu per animal of *Brucella melitensis* by injection intraperitoneal. Samples were collected over a 6 week period of infection. Blood collected for serological test revealed positive for animals were injected with bacteria then animal dissected, liver and spleen collected and kept in 10% formalin for histological study. **Results showed** Histopathological changes show congestion, granulomatous, fatty change, fibrosis in liver and increased number of lymphohistiocytic cells and increased amount of white pulp. The study concluded that the pathological changes in liver and spleen caused by brucellamelitensis in mice are similar to those observed in humans with brucellosis.

Introduction

Brucellosis is an infectious disease caused by bacteria of the genus *Brucella* that affect humans as well as domestic and wild animals, leading to significant impact on public health and animal industry. *Brucella spp.* Is a Gram negative, facultative intracellular bacterium that is able to survive and replicate in phagocytic and nonphagocytic cell, establishing a chronic infection in both humans and animals ⁽¹⁾. Human brucellosis is considered as a life-threatening debilitating disease characterized by weakness, fever, malaise, arthritis, osteomyelitis, endocarditis or meningoencephalitis ⁽²⁾. The disease is characterized by nonspecific symptoms, including undulant fever, weight loss, depression, hepatomegaly and splenomegaly, arthritis, spondylitis, osteomyelitis, epididymitis, and orchitis, as well as other more severe complications as neurobrucellosis, liver abscesses, and endocarditis, are also commonly described in patients ^(3,4). *Brucella melitensis*, is one of the six species of brucella, of which are known to be capable of infecting humans ^(5,6).

The mouse has been the most widely used brucellosis model. Mice were first used by Holth in 1911 for *Brucella* vaccine testing. Thereafter, mice were used for etiological confirmation of samples from infected animals, to test virulence and for the evaluation of the pathological lesions ⁽⁷⁾. The results in mice are not immediately applicable and transferable to humans or to the target animal species. However, the uncovering of a significant phenotype in mice using an appropriate protocol gives useful information ⁽⁸⁾.

Brucella infection may occur by digestive route, inhalation or through nasal mucosa or conjunctiva ^(9,10). After crossing the mucosal barrier, the organisms reach regional lymph nodes, replicate in macrophages, and establish a systemic and persistent infection. A bacteremia phase of infection results in colonization of the spleen, liver, and osteoarticular tissues, and depending on the *Brucella* species and host, it may also colonize the mammary gland and the reproductive system ^(9,10,11).

In murine models of *Brucella* sp. infection, experimental inoculation is performed mostly through three routes: intraperitoneal, digestive, or nasal (aerosol)

MATERIAL AND METHODS:

Animals and History:

The present study was carried out on a total number of 20 female mice (8 weeks of age) obtained

* Corresponding author at: University of Anbar-Collage of Medicine.

E-mail address:

from laboratory animals were distributed into two groups. Group A: 10 mice infected with *B. melitensis* and group B: 10 mice without infected as a control. Mice were kept in conventional animal facilities and received water and food ad libitum.

Isolation and Identification of Brucella:

Brucella was isolated from sheep abortion state and identification for Brucella according to the technique recommended by Alton et al.⁽¹²⁾. Bacteria was first grown onto Brucella agar under appropriate condition and was used for subsequent experimental infection of mice. Briefly, from Brucella agar, single colony of bacteria was transferred into 10 mL of Brucella broth and incubated at 37°C for 72h. The concentration of bacteria in the broth was adjusted to 0.5 McFarland turbidity standards and from which 1 mL, approximately containing 5×10^8 cfu was used to infect the mice intraperitoneally by the methods described previously Zerva et al.⁽¹³⁾. In addition, 10 mice, each injected with 1 mL of Brucella broth, and used as a negative control group. Samples were collected over a 3-4 week period of infection and kept in 10% formalin for histological study.

Sample collection:

After 120 days following exposure, 5 mice (group A) and two mice (group B as a control), liver and spleen were taken from brucella seropositive cases for bacteriological. The organs are prepared for pathological examination.

Histopathological Examination:

specimens included liver and spleen were collected and fixed in 10% formalin solution then wash, dehydrated, embedded in paraffin, sectioned at 4-5 micron thickness and stained with hematoxyline and eosin as a routine work for histopathological studies Bancroft & Stevens⁽¹⁴⁾.

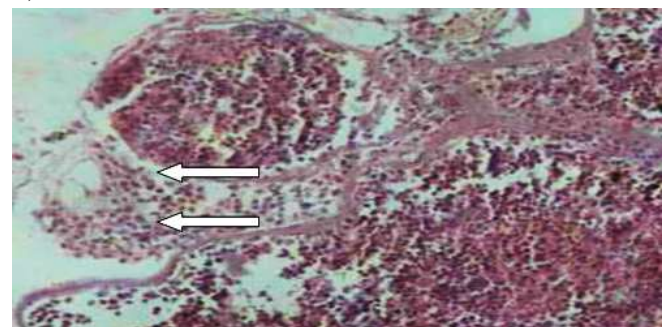
RESULTS:

Histopathological changes

During Brucella infection in the mouse, the spleen is the most heavily colonized organ, and it showed mild hyperplastic activation of the white pulp with the presence of abundant histocytic and plasma cells around the medullary cords of the red pulp (Fig 1). Active proliferation of reticulum cells was the characteristic picture in most cases. Epithelioid and

giant cell microgranuloma was also detected surrounded by the rem of lymphocytes and there are some of fibroblast cells (Figure 2).

The liver is also an important site for colonization and replication of Brucella in the mouse. Usually, mice infected with brucella have mild to moderate hepatitis, which is characterized by neutrophils infiltrate at early stages of infection (Fig 3), followed by histocytic infiltrate with epithelioid cells and microgranulomatous at chronic stages of infection with bacteria localizing intracellular in macrophages within microgranulomatous lesions (Fig 4).



Fig(1) histocytic and plasma cells around the medullary cords of the red pulp (15X)

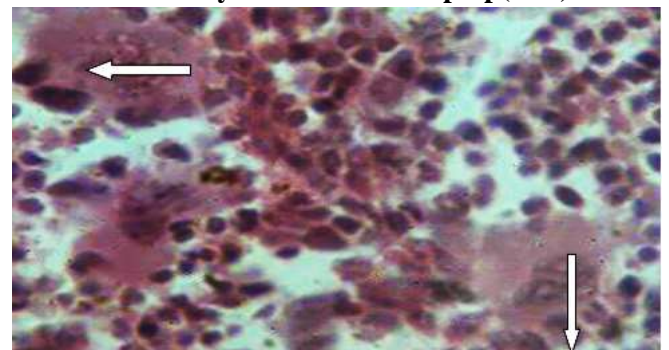
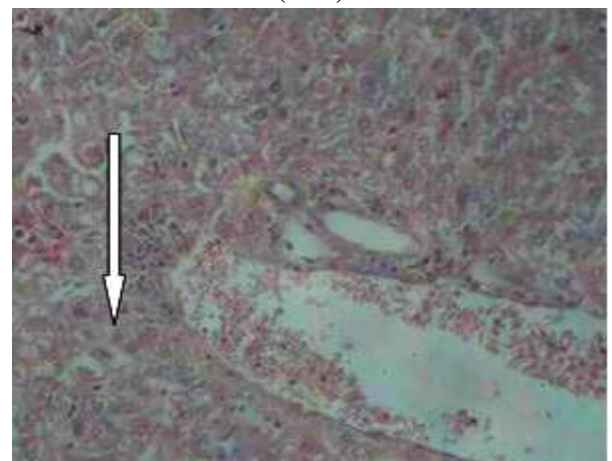


Fig (2) Epithelioid and giant cell microgranuloma. (40X)



Fig(3) Liver neutrophilic infiltration at early stages of infection. (40X)

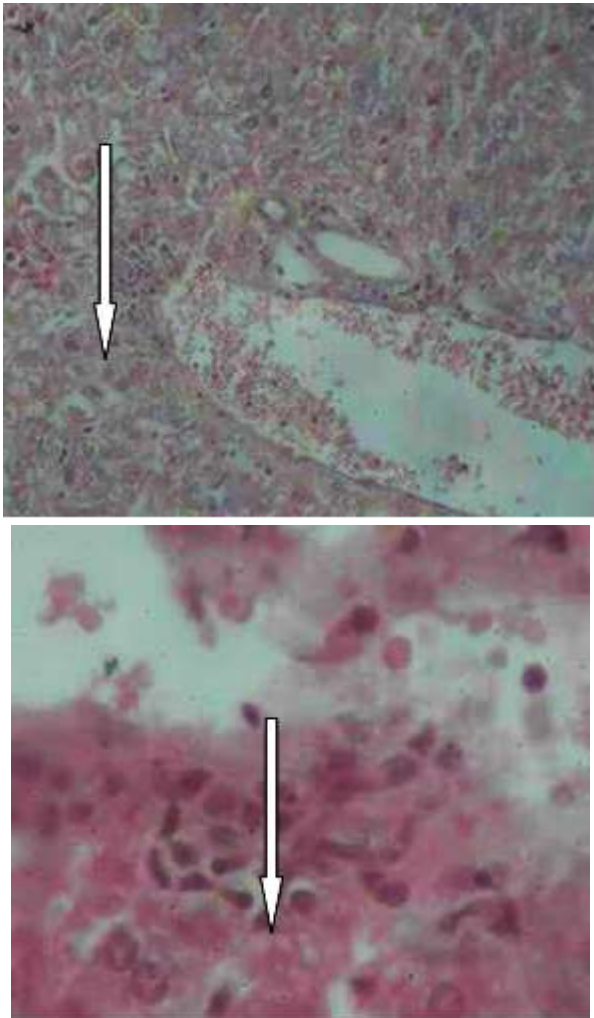


Fig (4) Liver microgranulomatous lesions. (15X)

Discussion:

Brucellamelitensis is the most invasive species and produces the most serious infection in human and animals⁽¹⁵⁾. Our study revealed the histological changes in liver and spleen and the role of brucella in damage of these organs and others else. The histopathological changes in the liver revealed degenerative changes associated with focal leukocytes infiltration. Young et al,⁽¹⁶⁾ reported that poorly formed hepatic granuloma, composed of leukocytes infiltration with or without necrosis, was demonstrated in mice infected with *brucellamelitensis*. The spleen showed mild hyperplastic activation of the white pulp with the presence of abundant histiocytes and plasma cells around the medullary cords of the red pulp. Active proliferation of reticulum cells was the characteristic picture in most cases. Epithelioid and giant cell granuloma was also detected. These results agree with El-Nesser et al.⁽¹⁷⁾. Mice intraperitoneally infected with *B. melitensis* develop significant splenomegaly, which is more prominent

than in mice infected by aerosol^(18,19). During brucella sp. Infection in the mouse, the spleen is the most heavily colonized organ, and it develop histiocytes infiltrates and multifocal microgranuloma^(20,21). The liver is also an important site for colonization and replication of brucella sp. In the mouse⁽²²⁾. Mice infected with virulent strains of brucella sp. Have mild to moderate hepatitis, which is characterized by neutrophils infiltrate at early stage of infection, followed by histiocytes infiltrate with epithelioid cells and microgranuloma at chronic stages of infection with bacteria localizing intracellular in macrophages within microgranulomatous lesion⁽²⁰⁾. In our study we are observed multifocal granulomas with epithelioid macrophages in the parenchyma of the liver and spleen in biopsy samples from infected patients that agree with Colmenero et al, and Akritidis et al^(23,24). The histopathological changes in the liver revealed degenerative changes associated with focal leukocytes infiltration that agree with Young et al⁽¹⁶⁾ reported that poorly formed hepatic granuloma, composed of leukocytes infiltration with or without necrosis, was infected with brucellamelitensis. Our study reported that the enlarged spleens and weight increased evident during necropsy were related to the brucella exposure that agreement with Mark et al⁽²⁵⁾.

The study concluded that the pathological changes in liver and spleen caused by brucellamelitensis in mice are similar to those observed in humans with brucellosis.

REFERENCE:

- 1- Franco MP, Mulder M, Gilman RH, and Smits HL. 2007. "Human brucellosis", Lancet infection diseases, vol.7, no.12. pp.775-786.
- 2- Paixao TA, Costa EA, Xavier MN, et al. 2009. Innate immunity in brucellosis. Res Adv infect immune; 1: 21-37.
- 3- Fugier E, Pappas G, Gorvel JP. 2007. Virulence factors in brucellosis: implications for aetiopathogenesis and treatment. Expert Rev Mol Med; 9:1-10.
- 4- Hartigan PJ. 1997. "Human brucellosis epidemiology and clinical manifestations". Irish veterinary journal, vol.50, no.3, pp.179-180.
- 5- Teane. Silva MA, Erica. Costa A, Tatiane . Paixao A, Renee . Tsolis M and Renate L. Santos. 2011. Laboratory animal model for brucellosis research.

- Journal of Biomedicine and Biotechnology. Article ID 518323,9 pages.
- 6-Mariana N, Xavier, Tatiane A, Paixao, Andreas B, Denlartigh, Renee M. 2010. Tonsils and Renate L. Santos. Pathogenesis of Brucellaspp.The open veterinary science Journal. 4:109-118.
- 7-Maria-Jesus Grillo, Jose Maria Blasco, Jean PierrGorvel, Ignacio Moviyon and Edgardo Moreno. 2012. What have we learned from brucellosis in the mouse model? Grilloetal.VeterinaryResearch ,43-29.Http:www.veterinaryresearch.orglcontentl4311129.
- 8-Mestas J, Hughes CC: 2004. Of mice and men : Differences between mouse and human immunology. J Immunol, 172:2731-2738.
- 9-Thoen CO, Enright F and Chevillle NF. 1993."Brucella" in pathogenesis of bacterial infections in animals,C.L.Gyles and C.O.Thoen, Eds.,pp.236-247, lowastate university press,Ames,lowa,USA,2nedition.
- 11-Neta AVC, Mol JPS, Xavier MN, Paixao TA, Lage AP, and Santos RL.2010."Pathogenesis of bovine brucellosis",veterinary journal, vol.184, no.3, pp.146-155.
- 12-Alton GG, Jones LM and Pietz DE.1975. Laboratory techniques in brucellosis 2 noted FAO/Wito,USA,pp: 11-59.
- 13-Zerva L, Bourantas K, Mitka S, Kansonzidou A and NLegakis NJ. 2001. Serum is the prepered clinical specimen for diagnosis of human brucellosis by PCR.J.Clin.Microbiol.,39:1661-1664.
- 14-Bancroft JD, and Stevens A. 1996. Theory and practice of Histological Technique.Churchillliveigstone,New York.
- 15-Hinic V, Brodard I, Thomann A, Holub M, Miserez R and Abril C. 2009. 15711 based real time PCR assay as atool for detection of brucellaspp.In wild boars and comporasion with bacterial isolation and serology. BMC.Vet.Res.,5:22.
- 16-Young EJ, Gomez C, Yawn D and Musher D. 1979. Compansion of brucellaabortus and brucellamelitensis infections of mice and their effect on acquired cellular resistance. Infant.Immun.26(2):280-685.
- 17-El-Nesser,Mahdy E, Halaby AS. and Deeb S. 2007. Pathological and immunohistochemical studies on Brucellamelitensis in cows. J. Vet. Med. Giza,55(1):275-292.
- 18-Kahl-McDonagh MM, Arenas-Gamboa AM, and Ficht TA. 2007."Aerosol infection of BALB/C mice with brucellamelitensis and b.abortus and protective efficacy against aerosol challeng" infection and immunity, vol.75, no.10, pp.4923-4932.
- 19-Stevens MG, Oslen SC, Palmer MV, and Pughjr GW. 1996. "Immune responses and resistance to brucellosis in mice .Vaccinated orally with brucellaabortus RBB51"infection and immunity , vol.64,no.11. pp.4534-4541.
- 20-Enright FM, Araya LN, Elzer PH, Rowe GE and Winter AJ. 1990."Comparative histopathology in BALB/C mice infected with virulent and attenuated strains of Brucellaabortus"Veterinary immunology and immunopathology,vol.26,no.2,pp:171-182.
- 21-Stevens MG, Olsen SC, Pughjr GW and Palmer MV. 1994."Immune and pathologic responses in mice infected with brucellaabortus 19.RB 51,or 2308" infection and immunity, vol.62.no.8, pp:3206-3212.
- 22-Izadjoo MJ, Mense MG, Bhattacharjee AK, Hadfield TL, Crawfad RM and Hoover DL. 2008."Astudy on the use of male animal models for developing a live vaccine for brucellosis"Transboundary and Emerging Diseases, vol.55,no.3-4,pp:145-151.
- 23-Colmenero JDD, Queipo-Ortuo MI, Reguera JM, Suarez-Mouz MA, Martin-Carballino S, Morata P. 2002."Chronic hepatosplenicabscessesinbrucellosis.Clinico-therapeutic features and molecular diagnostic approach" Diagnostic Microbiology and infections disease,vol.42, no.3,pp:154-167.
- 24-Akntidis N, Tzivras M, Delladetsima I, Stefanaki S, Moutsopoulos HM and G.Pappas G. 2007."The liver in brucellosis" Clinical Gastroenterology and Hepatology, vol.5, no.9, pp:1109-1112.
- 25-Mark G , Mense , DVM, PhD, MBA; Louisepitt M, PhD; David Hoover L, MD. 2004. Pathologic changes associated with Brucellosis experimentally induced by aerosol exposure in rhesus macaques (MaCacamulatta).Amj Vet Res;65:644-652,2004.

دراسة التغيرات النسيجية في كبد وطحال الفئران المصابة ببكتريا *Brucellamilitensis*

سميعة مجبل حمد عبير يوسف عبد الكريم نجم الدين عبدالله

الخلاصة:

تناول البحث دراسة تأثير الإصابة ببكتريا البروسيلا من جنس *Brucellamilitensis* ودرجة الضراوة لها على أنسجة كلا من الكبد والطحال فيالفئران.تضمنت الدراسة استخدام 20 من الفئران الإناث بعمر ثمانية أسابيع، 10 منها استخدمت مجموعة سيطرة و10 أخرى تمت معاملتها ببكتريا البروسلا حيث تم حقن 10^5 من عزلة نقية للبروسيلا في البريتون. حضنت البكتريا بالحيوانات لمدة ثمانية أسابيع بعدها تم تخدير الحيوانات وجمع عينات الدم للفحوصات السيرولوجية ثم شرحت الحيوانات وجمعت الأعضاء المنتخبة وتم تحضيرها الدراسة النسيجية . أظهرت النتائج وجود تغيرات واضحة في أنسجة كل من الكبد والطحال تمثلت بظهور احتقان وتغيرات دهنية وتليفات وغيره في الكبد مع تنخر وزيادة نسبة اللب الاحمر في الطحال مع تجمع للخلايا الالتهابية.