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Histopathological changes in liver and spleen of mice infected with brucellosis

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Abstract:

A total of 20 female mice (8 week of age)a 10 mice control and 10 deal with 10^5cfu per animal of *Brucella melitensis* by injection intraperitonial. 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 thatthepathological changes in liver and spleen caused by brucellamelitensis in mice are similar to those observed in humans with brucellosis

Key word:*Brucella,* liver, spleen.

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 nonphagocyticcell, 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, endocarditics ormeningoencephalitis[(2)]The disease is characterized by nonspecific symptoms,including undulantfever,weightloss. depression, hepatomegalyand, splenomegaly, arthritis,spondylitis,osteomylits,epididymitis,and orchitis, as well as other more severe complications as neurobrucellosis, liver abscesses, and endocarditis, are also commonly described in patients[(3,4)]Brucellamelitensis, 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 bydigestive route, inhalation or through nasal mucosa orconjunctiva [(9,10)]. After crossing the mucosal barrier,the organisms reach regional lymph nodes, replicate inmacrophages, and establish a systemic and persistent infection.A bacteremia phase of infection results in colonizationof the spleen, liver, and osteoarticular tissues, and dependingon the *Brucella*species and host, it may also colonize themammary gland and the reproductive system [(9,10,11)].

In murine models of *Brucella*sp. infection, experimentalinoculation 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 from laboratory animals were distributed into two groups. Group A: 10 mice infected with B.melitensis and group B: 10 mice without infected as acontrol.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 etal [(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 37C° for 72h.The concentration of bacteria in the broth was adjusted to 0.5 McFarland turbidity standards and from which 1 mL,approximately containing 5×10^8cfu was used to infect the miceintraperitonially by the methods described previouslyZerva 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 acontrol),liver and spleen were taken from brucellaseropositive cases for bacteriological. The organs are prepare for pathological examination.

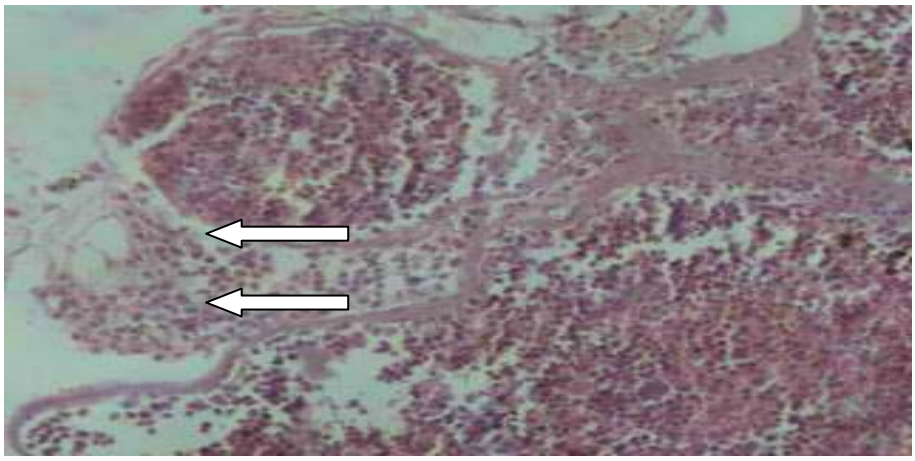
HistopathologicalExamination:specimens included liver and spleen were collected and fixed in 10% formalin solution then wash, dehydrated, embedded in paraffin, sectioned at 4-5 micron thickness and stained with hematoxyline and eosin as a routine work for histopathologicalstudies Bancroft&Stevens[14].

RESULTS:

Histopathological changes

DuringBrucella 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 (Fig1).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 localizingintracellular in macrophages within microgranulomatous lesions(Fig 4).



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

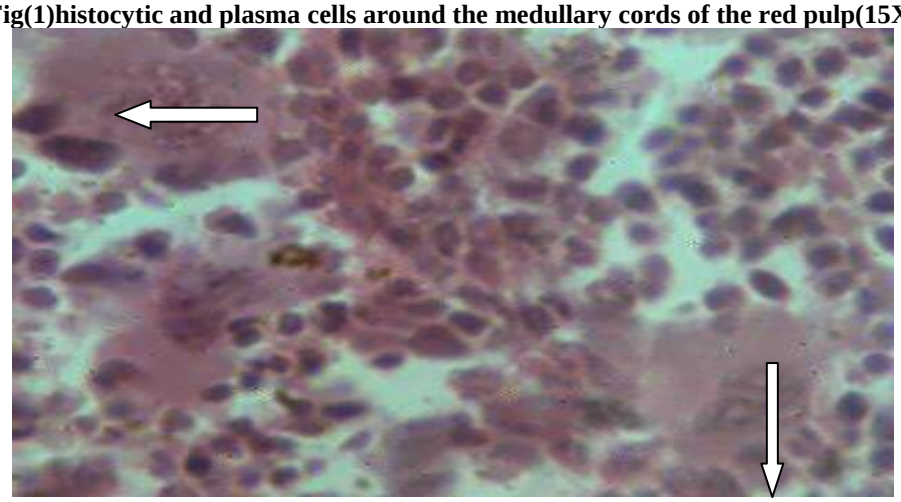
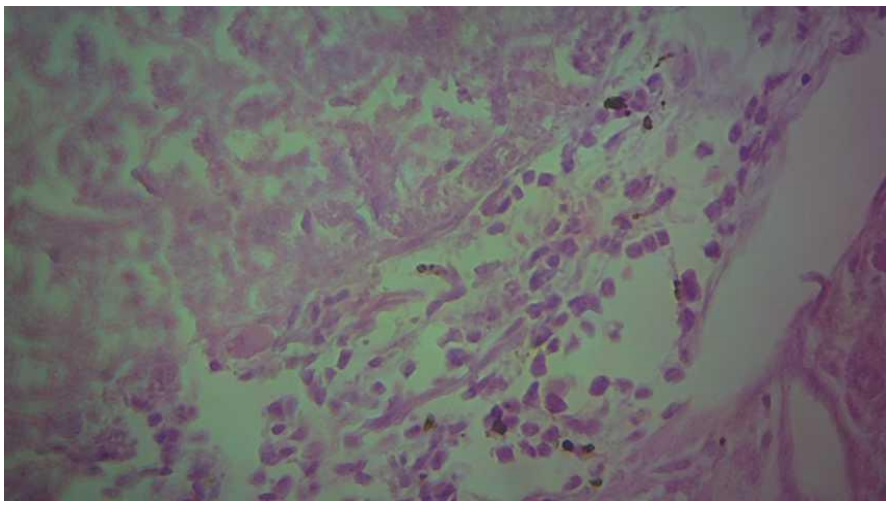


Fig (2)Epithelioid and giant cell microgranuloma. (40X)



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

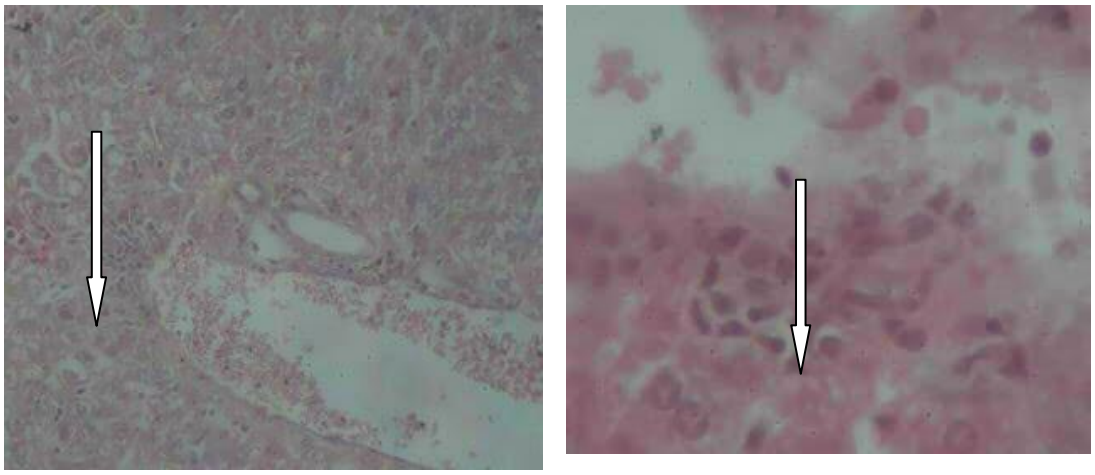


Fig (4) Liver microgranulomatous lesions. (15X)

Discussion:

Brucellamelitensis is the most invasive species and produces the most serious infection in human and animals[15].Our study are 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 andgiant cell granuloma was also detected. These results agree with El-Nesser et al.[17].Mice intraperitonially 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 neutrophilsinfiltrate at early stage of infection, followed by histiocytesinfiltrate with epithelioid cells and microgranuloma at chronic stages of infection with bacteria localizing intracellular in macrophages withinmicrogranulomatous 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

focalleukocytesinfiltration 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.Ourstudy reported that the enlarged spleens and weight increased evident during necropsy were related to the brucellaexposure that agreement with Mark et al [25]. .

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

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

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الخلاصة:

تناول البحث دراسة تأثير الإصابة ببكتريا البروسيلا من جنس *Brucellamilitensis* ودرجة الضراوة لها على أنسجة كلا من الكبد والطحال فيالفئران.تضمنت الدراسة استخدام ٢٠ من الفئران الإناث بعمر ثمانية أسابيع، ١٠ منها استخدمت مجموعة سيطرة و١٠ أخرى تمت معاملتها ببكتريا البروسلا حيث تم حقن 10^5من عزلة نقية للبروسيلا في البريتون. حضنت البكتريا بالحيوانات لمدة ثمانية أسابيع بعدها تم تخدير الحيوانات وجمع عينات الدم للفحوصات السيريولوجية 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 وتلييفات وغيره في الكبد مع تنخر وزيادة نسبة اللب الاحمر في الطحال مع تجمع للخلايا الالتهابية.