

ORIGINAL ARTICLE

Histopathological changes of brucellosis in experimentally infected guinea pig

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Abstract

Background: Brucellosis, a chronic infectious and zoonotic disease, is endemic in many countries of the world including Bangladesh, that affects humans and animals, leading to significant impact on public health and animal industry. There are several reports on seroprevalence, risk factors, molecular, epidemiological and review of brucellosis in human and animals but least of histopathological reports in Bangladesh. As laboratory animal species guinea pigs are the most susceptible to *Brucella* infection in comparison to mice, monkeys, rats and sheep. Hence, this study was undertaken to determine the histopathology of brucellosis in guinea pig.

Methods: After collection of aborted bovine foetal membranes from the Central Cattle Breeding and Dairy Farm (CCBDF), Savar, screened with modified Ziehl-Neelsen staining method and inoculum (10 CFU/2ml) prepared from positive samples were inoculated into each of ten guinea pig. The guinea pigs were sacrificed after showing positive results from the serum in Rose Bengal test and from blood in rapid kit test 3 weeks post inoculation. Then the gross and histopathological lesions were observed in the liver, spleen, heart, lungs, and kidneys.

Results: Liver showed congestion, haemorrhage and fatty changes and granuloma formation with infiltration of macrophages. However, only caseous necrosis of splenic follicle was observed in spleen. The major findings in lungs of guinea pig were granuloma, haemorrhage and necrosis of lung parenchyma and mononuclear cellular infiltration. The heart revealed haemorrhagic endocarditis and monocytes infiltration. A variable degree of multifocal accumulation of mononuclear cells infiltration and congestion were found in kidneys.

Conclusions: It is concluded that the histopathological changes caused by *Brucella* spp. are similar to those observed in humans with brucellosis. The animal models, particularly the guinea pig, can be used to study the detail pathogenesis of this organism.

Key words: Brucellosis, Guinea pig, Diagnosis, Histopathological Changes.

Introduction

Brucellosis, a chronic infectious and zoonotic disease, is endemic in many countries of the world including Bangladesh (Rahman *et al.*, 2017; Silva *et al.*, 2011) that affects humans and animals, leading to significant impact on public health and animal industry (Silva *et al.*, 2011). The Gram-negative bacteria infect a diverse array of land and aquatic mammals, including swine, cattle, goat, sheep, dogs, dolphins, whales, seals, and desert wood rats (Franco *et al.*, 2007; Hartigan, 1997) characterized by nonspecific symptoms, including undulant fever, weight loss, depression, hepatomegaly, and splenomegaly. Localized infections in various organs such as liver, spleen, mammary glands, uterus, epididymis, testis, etc. with histopathological changes in acute cases but osteoarticular, hepatobiliary, articular, spinal and neurological, or cardiovascular complications involves in chronic cases (Silva *et al.*, 2011; Corbel, 2006; Almuneef and Memish, 2003; Young *et al.*, 2014). Meanwhile in human brucellosis signs and symptoms include undulant fever, arthritis, spondylitis, endocarditis, meningitis and hepatosplenic abscesses in human (Tumwine *et al.*, 2015). Pathologically gross lesions are primarily observed in the reproductive tract though the uterus, including hemorrhagic placentomes with multifocal fibrinous exudation. Generalized lymphadenopathy, pleuritis and run-down appearance are also observed in advanced cases (Enright *et al.*, 1990; Preman *et al.*, 2013). Histopathologically hyperplasia and granulomatous lymphadenitis were observed in lymph nodes and necrotic debris in placentomes and several bacterial colonies surrounded by an intense inflammatory infiltrate (Nasruddin *et al.*, 2014). In mammary glands, liver, spleen and kidney were also marked with granulomatous inflammatory process. Multifocal or diffuse histiocytic meningitis in CNS, lymphoid depletion in thymus, lungs with diffusely thickened alveolar walls and interstitial inflammatory infiltrate were reported (Xavier *et al.*, 2010). The clinical signs of fever and malaise are non-specific, and the available serological diagnostic tests lack of high degree specificity in endemic regions compared to other important

public health diseases. A better understanding of the pathogenesis of brucellosis through discoveries in animal models could lead to improved diagnostics and potentially a vaccine (Hensel and Arena-Gamboa, 2018).

There are several reports on seroprevalence, risk factors, molecular, epidemiological and review of brucellosis in human and animals in Bangladesh (Islam *et al.*, 2013; Rahman *et al.*, 2016; Ahasan *et al.*, 2017; Rahman *et al.*, 2017). But least of histopathological reports are available in Bangladesh. As laboratory animal species guinea pigs are the most susceptible to *Brucella* infection and in early comparative studies of susceptibility in guinea pigs, mice, rats, and sheep demonstrated that they developed granulomatous lesions while inoculated with 10 CFU of *B. melitensis* or *B. suis* (Taran and Rybasov, 1971). Considerable lesions were observed in the liver, spleen, lungs, and lymph nodes, resembling those described in humans (Braude, 1951). Guinea pigs inoculated subcutaneously with infectious doses of *B. abortus*, *B. suis*, or *B. melitensis* develop a persistent bacteremia for 6 weeks after infection, whereas the attenuated *B. abortus* S19 is cleared from the blood at one week after infection (Cruickshank, 1957). Therefore, the guinea pig model was considered valuable for the evaluation of *Brucella* infection because of pathogenicity of all classic *Brucella* species to guinea pig (Garcia-Carrillo, 1990).

In this study, animal inoculation technique has been considered a better alternative for detecting *Brucella spp* from clinical specimens (Alton *et al.*, 1988). Hence, the objectives of this study were to detect the histopathology of brucellosis using guinea pig inoculation technique.

Methodology

Ethical approval

The study protocol was approved by Bangladesh Agricultural University Animal Welfare and Experimentation Ethics Committee (AWEEC/BAU/2019/17).

Sample collection

Aborted foetal membranes were collected aseptically and transferred in frozen condition from Savar to Mymensingh.

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Staining of impression smear

The samples were cut into pieces to expose the cutting surface and impression smear of the foetal membrane were stained by modified Ziehl-Neelsen staining method (Alton *et al.*, 1988), then dried and observed under microscope using 100x objective (oil immersion).

Guinea pig inoculation

The experimental guinea pigs were collected from ICDDRB (International Center for Diarrhoeal Disease Research, Bangladesh), Dhaka. The guinea pigs that were tested negative for brucellosis by RBT and rapid kit test were selected for inoculation. Ten guinea pigs were selected and 2 ml (10 CFU) of inoculum prepared from the positive samples by grinding was injected subcutaneously and blood was collected after 3 weeks.

Postmortem examination of guinea pigs and sample collection

The RBT and Genomix *Brucella* Antibody Detection Rapid test kit positive guinea pigs were sacrificed 3 weeks post inoculation. The suspected organs spleen, lymph node, liver and lungs were collected in 10% neutral buffered formalin for histopathology.

Histopathology

For histologic and morphologic analysis, tissue sections were processed in an automatic tissue

processor. Sections (5 µm thick) of paraffin-embedded tissues were then stained with routine haematoxylin and eosin (H&E) stain in accordance with standard protocols. The H & E-stained sections of tissue were examined by use of a microscope and color video camera. Histologic sections were assigned a score on the basis of relative severity of lesions (no lesions, minimal, mild, moderate, or severe), in which minimal lesions reflected only a slight change and severe lesions reflected extensive architectural involvement.

Results

Staining result

Numerous *Brucella* like gram-negative coccobacilli (short rods) organisms were observed under microscope in modified Ziehl-Neelsen staining.

Necropsy

Changes were observed in liver, spleen, heart, lungs and kidney. But more prominently the change was observed in spleen as splenomegaly but color unchanged. The liver and heart (endocardium) were congested.

Histopathological changes

The major histological changes were observed in liver, spleen, heart, lungs and kidney.

Table 1. Histopathological changes in different organ of guinea pig

Organ	Changes
Liver	Liver as a major organ of the mononuclear phagocytic system, is probably involved in all cases of brucellosis. The major findings were congestion, haemorrhage and fatty changes in the liver (Figure 1). Wide spread congestion and hemorrhages were seen in the hepatic tissue. Fatty changes and granuloma formation with infiltration of macrophages were also seen in liver.
Spleen	Histologically there was no major changes in spleen but caseous necrosis of splenic follicle was observed (Figure 2).
Lungs	The major finding in lungs of guinea pig were granuloma, haemorrhage and necrosis of lung parenchyma (Figure 3). Necrosis, wide spread hemorrhages and mononuclear cellular infiltration were seen in the lung tissue.
Heart	The heart revealed no pathological alterations except haemorrhagic endocarditis and monocytes infiltration (Figure 4). In the endocardium, a wide spread congestion and hemorrhages was seen microscopic fields.
Kidneys	The kidneys showed variable degrees of multifocal accumulation of mononuclear cells infiltration and congestion of the kidneys. (Figure 5). A greater infiltration of macrophages in the renal tissue were observed with wide spread congestion and hemorrhages.

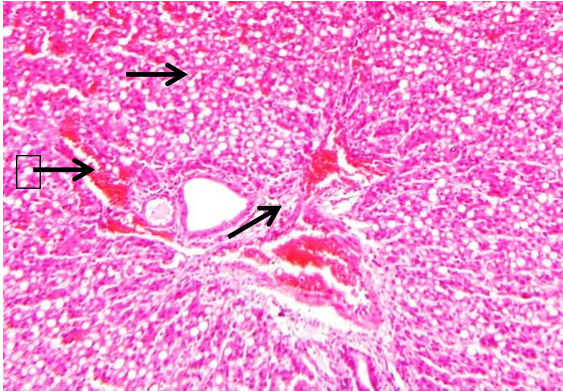


Figure 1: Section of the liver of guinea pig infected with *Brucella spp.* Wide spread congestion and hemorrhages (red arrow). Fatty changes and granuloma formation with infiltration of macrophages (black arrow)

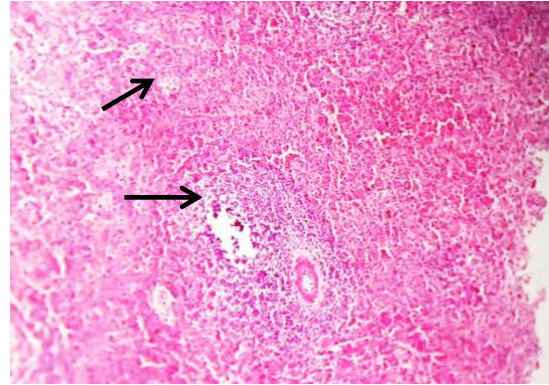


Figure 2: Section of spleen of guinea pig infected with *Brucella spp.* Active extramedullary hematopoiesis (red arrow) Caseous necrosis of splenic follicle (black arrow)

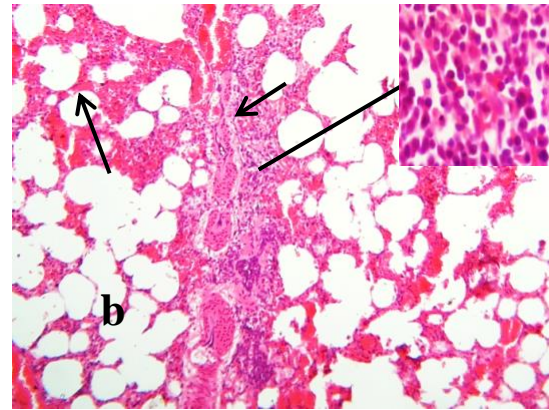
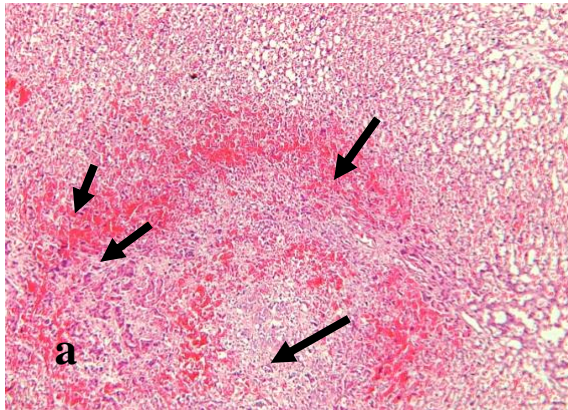


Figure 3: Section of lungs of guinea pig infected with *Brucella spp.* Necrosis (a, black arrow, 4x) and wide spread hemorrhages (a, red arrow) in the lung tissue (a, 4x). Wide spread hemorrhage (b, red arrow) and mononuclear cellular infiltration (inset) (black arrow, 40x).

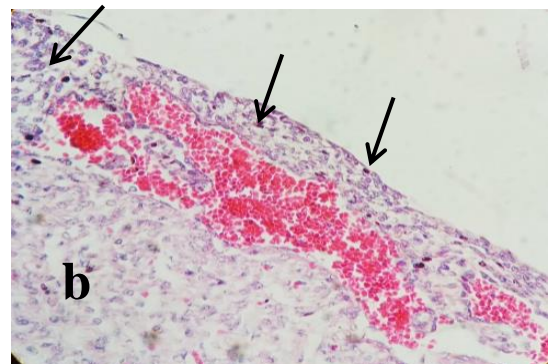
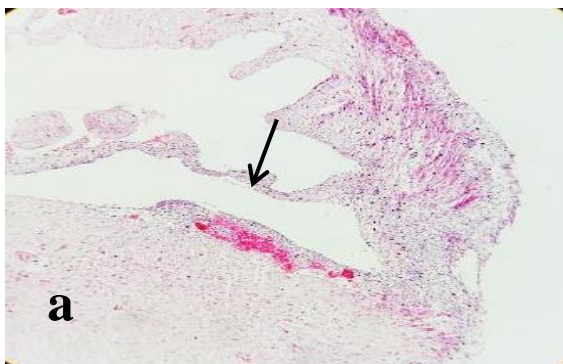


Figure 4: Section of heart of guinea pig infected with *Brucella spp.* Wide spread congestion and hemorrhages (a and b, red arrow) was seen in the endocardium both at low (a, 10x) and high (b. 40x) power microscopic fields. At higher magnification (b. 40x) mononuclear cellular infiltration was seen in the endocardium (b, black arrow).

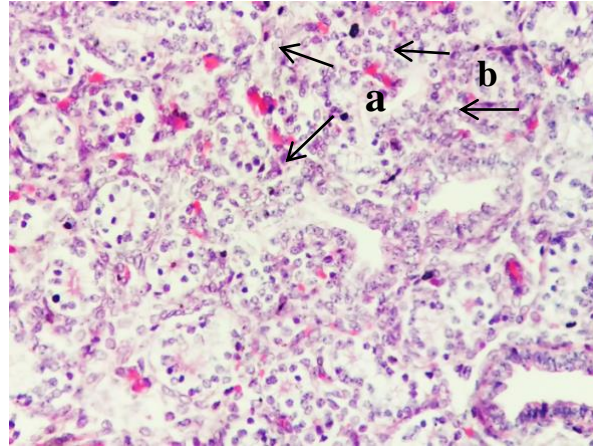


Figure 5: Section of a kidney of guinea pig infected with *Brucella spp.* Wide spread congestion and hemorrhages (a and b, red arrow) in the renal tissue (40x) with infiltration of macrophages (black arrow).

Discussion

This study was conducted mainly to focus on histopathologic lesion in guinea pig inoculated with *Brucella spp.* due to similarities of disease development and biologically relevant models that can improve understanding of pathogenesis. Histopathology in liver, heart, kidney, lung and/or spleen of all animal were analogous to human signs while increased inflammatory response and splenomegaly were confirmed in brucellosis infection (Henning *et al.*, 2012). The major histologic changes in guinea pigs were observed in liver, kidney, lungs and spleen but minor changes in heart which supported RMs model in case of brucellosis (Henning *et al.*, 2012). As a major phagocytic organ different histologic patterns can be observed in liver involvement in brucellosis, the most common being granuloma formation (Young *et al.*, 2014). The changes in liver was confirmed with wide spread congestion, haemorrhage in the hepatic tissue, fatty changes along with granuloma formation and infiltration of macrophages. Major changes in the liver were congestion, haemorrhage and fatty changes. Few changes in liver were reported by granuloma formation in the hepatic parenchyma, portal space with varying degrees of cellular infiltration along with parenchymal necroses and Kupffer's cell

hypoplasia and histiocytic inflammation with necrotic hepatocytes surrounded by neutrophils and macrophages (Hensel and Arenas-Gamboa, 2018; Akntidis *et al.*, 2007). But portal lymphocytic inflammation might be present (Young *et al.*, 2014).

Spleen is the most colonizing organ (Enright *et al.*, 1990). In spleen, splenomegaly with caseous necrosis of splenic follicle and active hematopoiesis were found which was agreed by others (Akntidis *et al.*, 2007). But hyperplasia of white pulp was predominating in the spleen of most cases and depletion of lymphocytes was also seen in the white pulp of spleen and cortex of lymph nodes (Hosein *et al.*, 2018).

In lungs the major findings were granuloma formation, necrosis, wide spread hemorrhages in the lung tissue and mononuclear cellular infiltration. Pathological analysis revealed alveolar wall thickening, telangiectasia with hyperemia, inflammatory cell infiltration, large areas of congestion and bleeding, and areas of focal necrosis (Fu *et al.*, 2018). No major changes were observed in heart bur endocardium was marked with haemorrhagic endocarditis and mononuclear cellular infiltration. The heart revealed no pathological alterations except

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myocarditis and necrotic changes in single instance (Hosein *et al.*, 2018). But in the absence of concomitant endocarditis, development of myocarditis is a highly rare complication of brucellosis (Lagadinou *et al.*, 2019).

In kidneys, congestion, haemorrhage and multifocal accumulation of mononuclear cells were observed. But there might be variable degrees of glomerular and tubular nephrosis. Liver, kidneys, lungs and heart, showed non-granulomatous reactions varied from mild vacuolar degeneration, early necrotic changes (pyknosis) to mild inflammatory reactions (focal mononuclear cell infiltration) (Hosein *et al.*, 2018). In some instances musculoskeletal, genitourinary, gastrointestinal, hematologic, nervous, skin, and mucous membranes and respiratory complications have been reported in several cases of brucellosis (Lagadinou *et al.*, 2019).

Therefore, the clinical specimen like aborted content might contain lot of contaminants and from such sample guinea pig inoculation method should be the best choice for the isolation of *Brucella* (Alton *et al.*, 1988). Before inoculation of foetal content to the guinea pig must be screened initially by Modified Ziehl-Neelsen technique. The pink colored coccobacilli in stained smear indicates probable presence of *Brucella* organism. The stain positive samples should be inoculated in guinea pig for expected histopathological changes and negative might be inoculated for control.

This histopathological study for the first time in Bangladesh using guinea pig inoculation techniques, reports the successful detection of pathological changes in major organs after inoculation with aborted fetal contents.

Conclusions

This result showed microscopic evidence of involvement of different organ and changes due to *Brucella* infection. This is more relevant in confirming the species and to avoid any cross reactivity of serological test like RBT or ELISA. It can be concluded that the histopathological

changes caused by *Brucella spp.* are similar to those observed in humans with brucellosis. The animal models, particularly the guinea pig, can be used and allowed for accumulation of valuable information of pathogenesis of *Brucella spp.* in vivo.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgement

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