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RESEARCH ARTICLE

Pathological changes in some internal organs of mice infected experimentally with *Salmonella mbandaka*

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Abstract

Salmonella is an important food borne pathogen worldwide. The purpose of the this study was to investigate the pathological changes occurred in the internal organs of infected mice with *Salmonella mbandaka*. Thirty mice with age range (6 – 8) weeks old of both sexes were divided randomly into two groups: first group (15 mice) inoculated with infective dose (ID) (1.3×10^7 CFU) orally and second group considered as a control group administrated 0.5ml PBS. Three mice from each group was sacrificed at 1 week, 2, 4, 6 and 8 weeks post inoculation(PI). Results At 1 &2 weeks post infection revealed goblet cells hyperplasia and hypertrophy with PMNCs infiltration together with coagulation necrosis of liver parenchyma and lymphoid depletion of spleen in evidence of interstitial nephritis also recorded,. While the characteristics manifestations during 4, 6 & 8 were lymphoid hyperplasia of splenetic tissue in addition to presence of granulation lesion in hepatic tissue.

In conclusion, this study revealed a different pathological changes in liver, spleen and kidney of mice infected with *S. mbandaka* , this indicate the ability of *S. mbandaka* to invade internal organ and replication in these organs.

Introduction

Salmonella species are Gram-negative, facultative intracellular bacteria that are distributed worldwide. *Salmonella* include two species, *S. enterica* and *S. bongori*. Humans are infected with *S. enterica* though contaminated food and water and present with a range of acute symptoms including gastroenteritis, headache and fever. Although systemic infections with *S*. Typhi are uncommon in developed countries, typhoid remains a significant public health problem in the developing world . Non-typhoidal Salmonella represents an important human and animal pathogen world-wide. Most human salmonellosis are food borne **(**Voetsch et al; (2004) but each year infections are acquired through direct or indirect animal contact in homes, veterinary clinics, zoological gardens, farm environments or other public, professional or private settings (Hoelzer et al;2011). *Salmonella enteric* serovars cause a variety of diseases ranging from self-limiting gastroenteritis to severe systemic infections .Virulence of these facultative intracellular pathogens is dependent on their ability to invade and replicate within non-phagocytic cells and have been studied experimentally using animal infection models in mice, cattle, and chickens (DeLeo and Otto, 2008; Santos et al,2001; Tsolis et al,1999; Wigley,2004**)**. Bacteria use virulence factors to induce intestinal inflammation, these factors liberates nutrients for selective use by the infecting microbe (Bliska and Van der Velden 2012). Intracellular *Salmonella* present in immune cells, e.g. macrophages and dendritic cells, may facilitate systemic infection by carrying the microorganism from the intestinal tract throughout the whole body, dendritic cells are important migratory phagocytes that are widely distributed throughout the body in lymphoid and non-lymphoid tissues **(**Sundquist et al; 2004).

Salmonella mbandaka were distributed worldwide, in Iraq was isolated at first time from stool samples of children suffered from diarrhea (Al-Talib,2011).

In Iraq, data regarding the use of this species as a model of *Salmonellosis* in animal is very scarce. Therefore, this work aimed to study and investigate the pathological changes of S*almonella mbandaka* in internal organ of mice .

Material and methods

1:bacteria :

Salmonella mbandaka was isolated from feverish child with severe diarrhea, dehydrated, this bacteria identified according to Quinn et al;(2004) and serotyped in National Center of *Salmonellae* in Baghdad.

2: laboratory animals (Mice):

Seventy eight mice,(BALB/c) mice of both sexes with age range (6–8) weeks old, obtained from the (National Center of Researches and Drugs Monitor in Baghdad) then adapted for two weeks before started experiments, they were fed on *ad libitum* commercial assorted pellets and clean water were used. Divided as follows, forty eight mice were used for estimation of the infective dose of *S. mbandaka* according to (Yousif and AL-Naqeeb,2010; Shallal 2011). And thirty mice were used for pathological study post infection, these mice were divided equally into 2 groups. First group was inoculated 0.5 ml orally containing 1.3×10^7 C.F.U. /ml of *S. mbandaka* and the second group administrated 0.5 ml of PBS .

Histopathology : -Three mice From each group were sacrificed by neck dislocation at 1, 2, 4, 6 and 8 post infection. Organs were removed under aseptic conditions and kept in 10% buffered formalin for 24 hours. Then, routine histopathological process was performed to obtain slides stained with haematoxylin and eosin (H&E) for histological evaluation (Bancroft et al;1994).

Ethical improvment

This study was approved by the ethical and research committee of Veterinary Medicine College/University of Baghdad.

Results and discussion

The pathological examination of organs infected with *S.mabandaka*, after 1 week was characterized by diffused and focal coagulation necrosis of liver parenchyma enclosed by thick inflammatory zone consists of PMNs together with atrophy of remaining hepatic cells as well as sinusoidal dilation (Figure. 1). In another section present focal necrosis associated with MNCs infiltrate mainly around portal area and marked dilatation of blood vessel. Also the results of liver showed appearance of basophilic masses of bacterial colonies at the border of coagulative necrotic area that appear more eosinophilic (Figure.2). Spleen showed slight lymphoid depletion in white pulp (Figure.3).Acute cellular swelling with cellular infiltration around congested blood vesicles, in another section of kidney showed focal MNCs cellular infiltration in the interstitial to gather with congestion and active hyperemia(Figure. 4), in another section of kidney showed sever dilation of renal tubules with cellular debris their lumen. Also the results of kidney marked sever dilation of renal tubules with vacuolar degeneration of epithelial lining of some tubules to gather focal interstitial MNCs aggregate While in the heart section there was separation of muscle by edema associated with slight cellular infiltration (Figure.5).

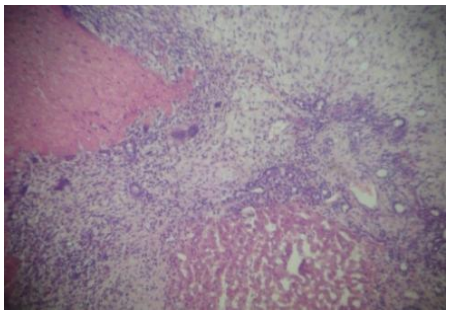

Figure 1: Photomicrograph of liver of mouse infected with *S.mbandaka* at 1 week PI shows shows diffused coagulation necrosis with atrophy of remaining Hepatic cells as well as sinusoid dilation. (H&E 20X).

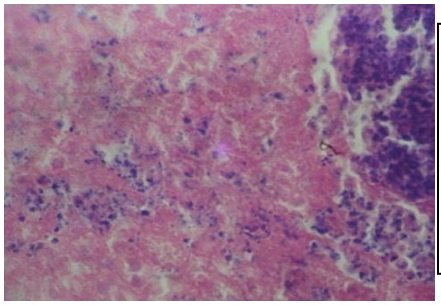

Figure 2: Photomicrograph of liver of mouse infected with *S.mbandaka* at 1 week PI shows basophilic masses of bacterial colonies surrounded by PMNCs infiltrate seen in esoinophilic necrosis areas(H&E 40X).

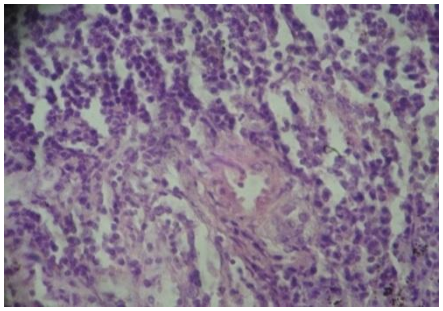

Figure 3: Photomicrograph of spleen of mouse infected with *S.mbandaka* at 1 week PI shows slight lymphoid depletion of interlobular septa In white pulp(H&E 40X).

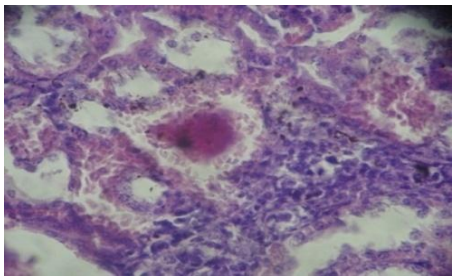

Figure 4: Photomicrograph of kidney of mouse infected with *S.mbandaka* at 1 week PI shows sever MNCs cellular infiltration in the interstitial together with congestion and active hyperemia (H&E 40X).

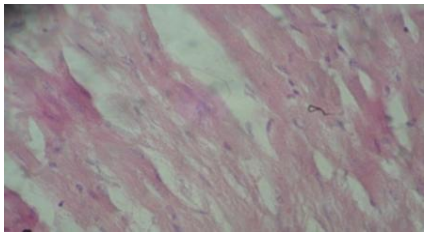

Figure 5: Photomicrograph of heart of mouse infected with *S.mbandaka* at 1 week PI shows intermuscular edema associated with slight cellular infiltration and congestion of blood vesicle (H&E 40X).

Post two week of infection, the liver showed intense MNC leucocytes aggregation in portal areas associated with sever proliferation of bile ducts with slight proliferation of kupffer cells (Figure. 6),In another section there was multi focal PMNs aggregate in liver paranchyme accompanied with appearance some necrotic and apoptotic cells(Figure. 7). The heart lesion was similar to above at 2 weeks moreover there was sever congestion and hemorrhage associate with edema and cellular infiltration between degenerated cardiac muscle , sever MNCs infiltration between hyalinized muscle bundles and the surround adipose tissue with evidence of myocarditis (Figure. 8).

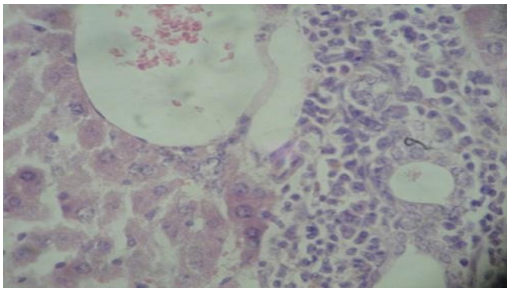

Figure 6: Photomicrograph of liver of mouse infected with *S.mbandaka* at 2 week PI shows intense aggregation of MNCs with sever congestion and dilatation of blood vesicles and sinusoid together with proliferation of bile duct. (H&E 40X).

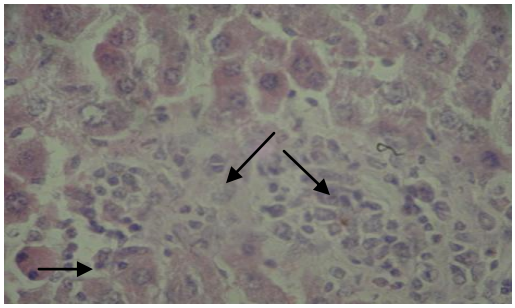

Figure 7: Photomicrograph of liver of mouse infected with *S.mbandaka* at 2 week PI shows multi focal MNCs aggregate with some necrotic and apoptotic cells ⟶
(H&E 40X).

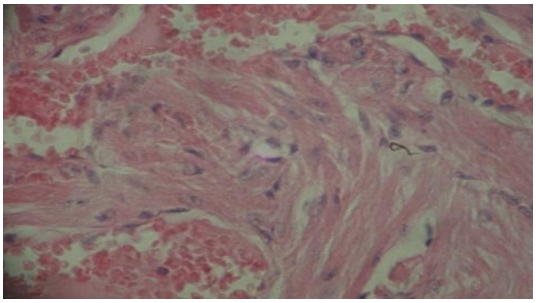

Figure 8: Photomicrograph of heart of mouse infected with *S.mbandaka* at 2 week PI shows sever congestion with edema, sever MNCs infiltration between hyalinized muscle with evidence of myocarditis(H&E 40X).

Post four weeks the liver showed individual necrosis of hepatocytes area with appearance number of apoptotic cells together few scattered cellular infiltration in dilated sinusoid (Figure.9). Slight lymphoid hypertrophy lymphoid atrophy with congestion in splenic red pulp (Figure. 10), in another section showed increase number of megakaryocyte with MNC infiltration in red pulp (Figure. 11). In kidney there was interstitial nephritis with diluted renal tubules togather with hyaline cast MNCs infiltration between tubule with necrosis of some tubule, also marked perivascular MNCs infiltration associate with vassal congestion infiltration between tubule with necrosis of some tubule.

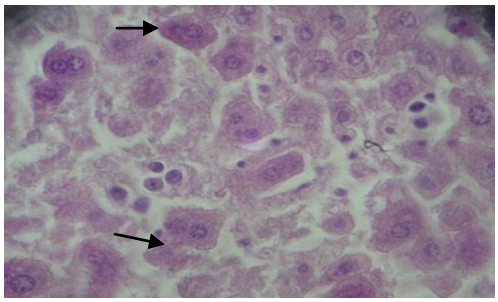

Figure 9: Photomicrograph of liver of mouse infected with *S.mbandaka* at 4 week PI shows MNCs infiltrate in dilated sinusoid (H&E 40X).

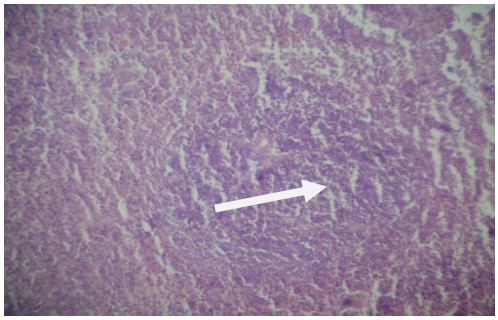

Figure 10: Photomicrograph of spleen of mouse infected with *S.mbandaka* at 4 week PI shows slight hyperplasia of lymphoid tissue with congestion in splenic red pulp(H&E 20X).

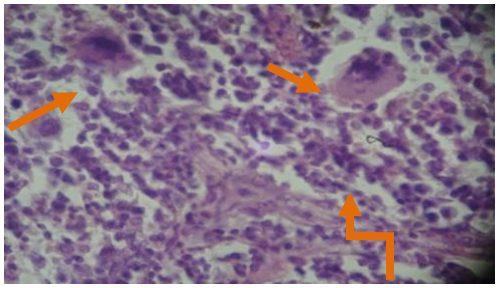

Figure 11: Photomicrograph of spleen of mouse infected with *S.mbandaka* at 4 week PI shows increase number of megakaryocyte with MNCs infiltrate in red pulp. (H&E 40X).

At six week of infection, liver investigate multiple granulomatous like reaction seen in liver paranchyme consist mainly of macrophage(Fig. 12) with sever necrosis of hepatic tissue. Spleen showed increase number of megakaryocytewith infiltration of inflammatory cells(Figure. 13) and active lymphoid hyperplasia in white pulp.The result of kidney marked granulomatous like reaction in the renal tissue seen mainly in periglomaruli (Figure. 14) accompanied with tubular necrosis with MNCs infiltration consist mainly of macrophages.

The histopathological examination of infective animals sacrificed after 8 weeks PI showed no clear pathological changes in most organs except spleen showed marked lymphoid hyperplasia with thick end central arteriole due to muscular hyper atrophy of its wall (Figure. 15) together with thick end trabecular and presence of cellular infiltrate with congestive in splenic capsule.

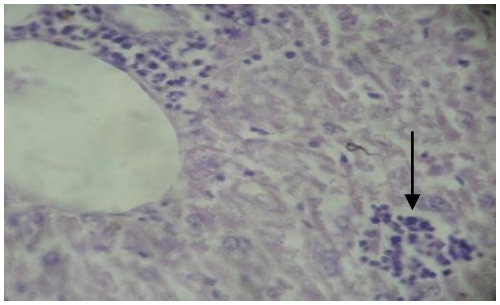

Figure12: Photomicrograph of liver of mouse infected with *S.mbandaka* at 6 week PI shows multiple grunulomatous lesions consist mainly of macrophage(H&E

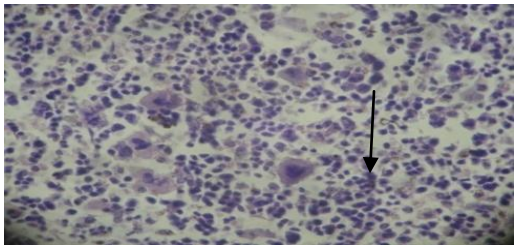

Figure 13: Photomicrograph of spleen of mouse infected with *S.mbandaka* at 6 week PI shows increase number of megakaryocyte ( ) (H&E 40X).

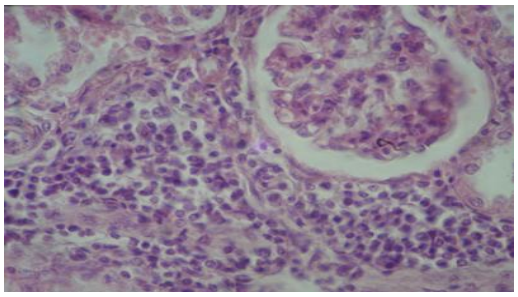

Figure 14: Photomicrograph of kidney of mouse infected with *S.mbandaka* at 6 week PI shows large granulomatous lesion seen mainly around glomeruli. (H&E 40X).

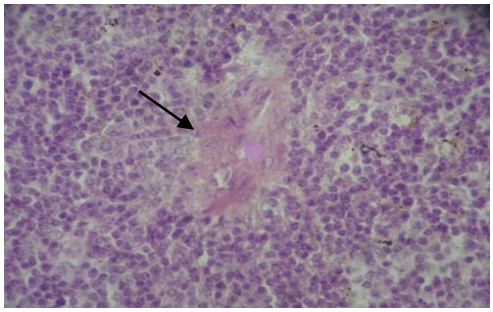

Figure 15: Photomicrograph of spleen of mouse infected with *S.mbandaka* at 8 week PI shows lymphoid hyperplasia with muscular hyper atrophy of splenic arteriole. (H&E 40X).

Salmonella mabandaka revealed significant patholological changes mainly during 1st and 2nd weeks in the internal target organs of experimental infected mice mainly in liver and this evidence agreement with study by (Bliska and Van der Velden, 2012). Also the results of liver showed appearance of basophilic masses of bacterial colonies at the border of coagulative necrotic area that appear more eosinophilia surrounded by cellular infiltrate are in agreement with studies of (Watson and Holden, 2010) they recorded the dissemination of bacterial pathogens in their hosts and on the processes that underlie bacterial spread and growth during organ colonization. They explained human typhoid fever caused by *Salmonella* enteric serovar*Typhimurium on mice*. which revealed the use of several routes of systemic dissemination that result in colonization and growth within the spleen and liver, the major sites for proliferation of bacteria.These approaches have provided more detailed insights into the events underlying the dynamics of Salmonella replication, spread and clearance within host organs and tissues.

The pathological pictures during 4-6 weeks reveals more changes ranged between lymphocytic hyperplasia splenic tissue to presence granunlomatous lesion mainly in the hepatic tissue and this evidence agreed with (Nesterenko et al; 2012) that mentioned the course of chronic Salmonellosis in susceptible I/St in bred line mice was characterized by the presence of more pronounced pathomorphologic changes in spleen and significantly higher microbial load in organs (approximately by 1000 times) , with pronounced splenomegaly and high concentration of *Salmonellae*. On the contrary A/Sn mice demonstrated a higher level of salmonella specific IgA forming cells in Peyer patches that probably leads to protect tion of A/Sn line during per oral infection. Genetic analysis of susceptibility to Salmonellosis. Also previous observation by (Fantuzzi et al,2011; Sheppard et al;2003) they showed small and diffuse foci of mixed inflammatory infiltrate in hepatic parenchyma. in contrast to Histopathological examination revealed by (Lima-Filho et al; 2010) that showed necrosis and inflammatory infiltrates were present in the spleen at approximately 10^4 cells/g of organ 28 days after challenge. However, no bacteria were detected in the liver at this stage

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