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Original Article

Histopathological changes in the Intestine and lung of mice infected experimentally with *Salmonella mbandaka*

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Abstract

Salmonella mbandaka has been isolated and identified from human in Iraq. The purpose of the present study was to investigate the histpathological changes in the internal organs of mice experimentally infected with *Salmonella mbandaka*. Thirty mice of both sexes with age range (6 – 8) weeks old were divided randomly into two groups: "group A" (15 mice) inoculated orally with infective dose (ID) (1.3×10^7 cells) and "group B" (15 mice administrated orally with 0.5 ml PBS) and considered as a control group. Both infected and non infected mice were Sacrificed after 1 week ,2 ,4 ,6 and 8 weeks post inoculation. After 1 &2 weeks post infection , results revealed a slight desquamation of intestinal mucosal epithelia together with tissue debris accumulated in lumen accompanied by hyperplasia and hyper atrophy of goblet cell, sub mucosal edema accompanied with blood vessels congestion surrounded with intense cellular infiltration. PMNs infiltration mainly in mucosa and sub mucosa of intestine and around bronchi associated with congested blood vessels in lung. While the characteristics manifestations during 4, 6 & 8were lymphoid hyperplasia of intestine tissue together with MNC pervious aggregation in lung. In conclusion, this study revealed a different changes in organs of mice infected with *S. mbandaka* , this indicate the virulence of this bacteria to cause a disease in mice and its ability to invade and replicate in intestine and lung.

Keyword : *Salmonella mbandaka*, *histopathology*, *Salmonella* infection.
Abbreviations: PMN= polymorph nuclear cell; PI= post infection, CFU= colony forming unit, MN= mononuclear cell, PBS= phosphate buffer saline

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Introduction

Salmonella mbandaka was distributed worldwide in human and animals (Hayward *et al* 2013; Le Doare *et al* ,2013). The microbiota of the mammalian intestinal tract represents a formidable barrier to colonization by pathogens. To overcome this resistance to colonization, bacterial pathogens use virulence factors to induce intestinal inflammation, which liberates nutrients for selective use by the infecting microbe (Bliska and Velden, 2012). Systemic infections represent severe manifestations of salmonellosis. 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). , in Iraq, S*almonella mbandaka* was isolated at first time from stool samples of diarrheal children by (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 histpathological effect of S*almonella mbandaka* in intestine and lung organ of mice.

Material and methods

Bacterial isolates

Salmonella mbandaka was obtained from zoonosis laboratory –College of Veterinary Medicine-University of Baghdad. Diagnosis was confirmed according to Quinn *et al.* (2004) and serotyping was done in the Central Public Health Laboratories (National Center of *Salmonellae* in Baghdad). The infective dose of *S. mbandaka* is (1.3×10^7 cells) was estimated according to (Shallal, 2011; Yousif & Al-Naqeeb, 2010).

Laboratory animals

Thirty mice (BALB/c) of both sexes, 6–8 weeks old, obtained from the National Center of Researches and Drugs Monitor in Baghdad. Before starting the experiment, the mice were adapted for two weeks by rearing in separated clean and disinfected cages, fed on commercial assorted pellets and clean water was supplied by *ad libitum*. Then, the mice were divided randomly into 2 groups. Group A was given orally 0.5ml (containing of 1.3×10^7 CFU/ml) of *S. mbandaka* while group B administrated 0.5 ml of PBS, and acted as controls.

Histopathological studies

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

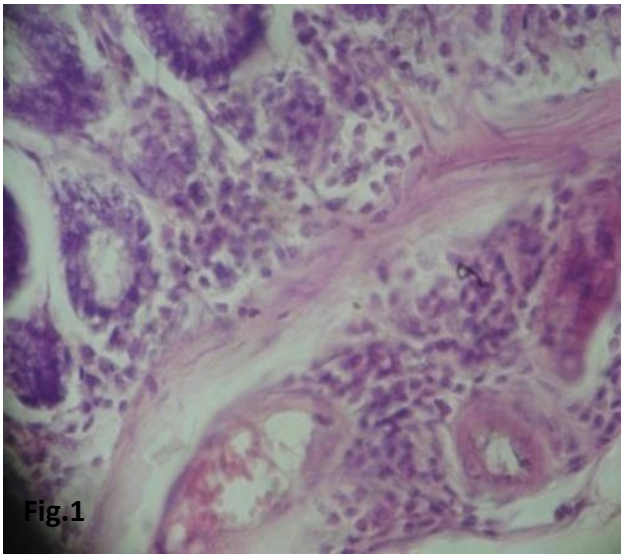
ISSN 2307-8073

Ethical approval

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

Results and discussion

The histopathological examination of organs infected with *S.mabandaka*, 1 week PI was characterized by slight desquamation of intestinal mucosal epithelia together with tissue debris accumulated in lumen accompanied by PMNs infiltration mainly in mucosa and sub mucosa of intestine, hyperplasia and hyper atrophy of goblet cell, sub mucosal edema accompanied with blood vessels congestion surrounded with intense cellular infiltration (Figure.1), and sub epithelial PMNs and MNCs with degeneration and necrosis of mucosal gland were frequently observed. The lung histolesions showed severe PMNs infiltration around bronchi associated with congested blood vessels, active alveolar hyperemia with sloughing of bronchial epithelia (Figure.2).



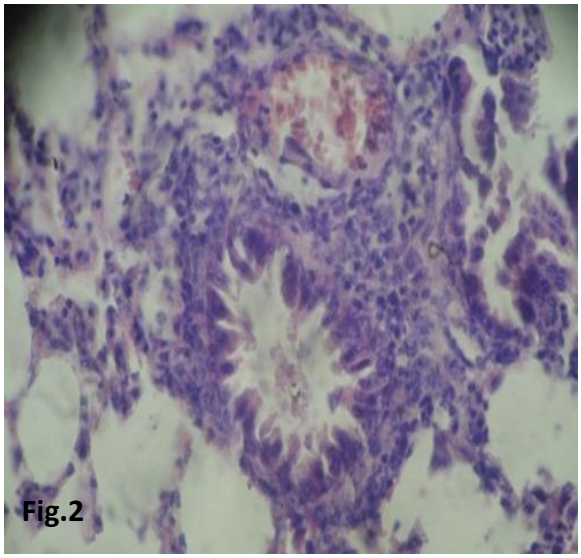


Figure 1: Photomicrograph of intestine of mouse infected with *S.mbandaka* at 1 week PI shows sub mucosal edema accompanied with MNCs aggregation and congestion blood vesicles(H&E 40X) .
Figure 2: Photomicrograph of lung of mouse infected with *S.mbandaka* at 1 week PI shows cellular aggregation a round bronchi &b.v with sloughing of bronchial epithelial (H&E 40X).

The characteristic manifestation of infective animals sacrificed after 2weeks post infection with S. mbandaka characterized by slight cellular infiltration with sever hyperplasia mucosal gland of intestine (Fig. 3). The lung showed presence of suppurative bronchopneumonia in which intense neutrophil infiltration in bronchiol & acinar tissue with bronchiectasis accompanied with mucopurulent exudate in their lumen to gather with fibro muscular hyperplasia of bronchiolar wall (Fig. 4).

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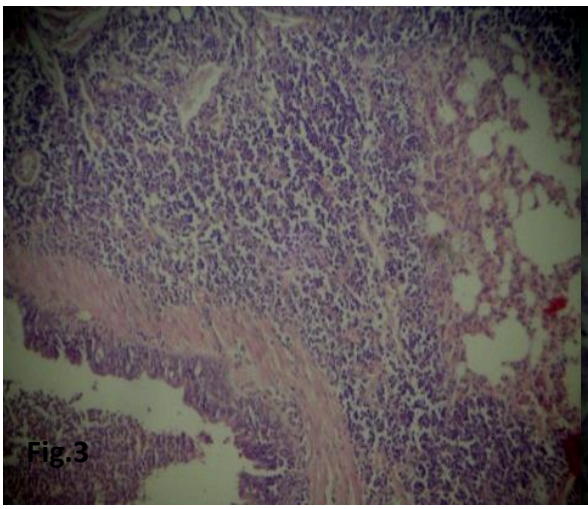

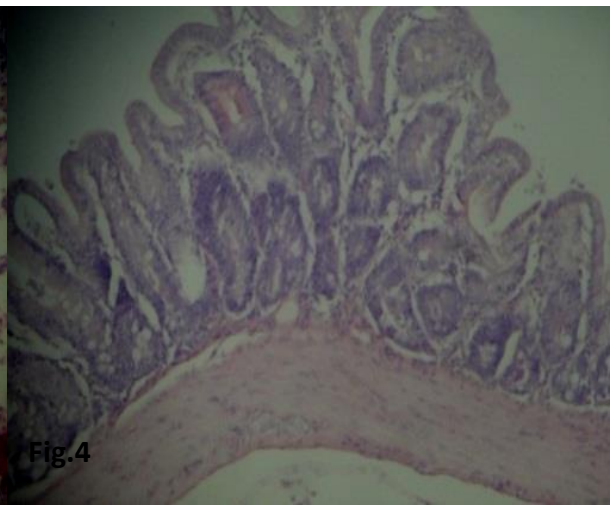

Figure 3: Photomicrograph of intestine of mouse infected with *S.mbandaka* at 2 week PI shows slight cellular infiltration with shortening of mucosal villi. (H&E 40X).

Figure 4: Photomicrograph of lung of mouse infected with *S.mbandaka* at 2 week PI shows suppurative bronochopn accompanied with bronchiectasia (H&E 40X).

After 4 weeks post infection with S.mbandaka, the scarified mice showed intense lymphocytic aggregate in the sub mucosa that appear as nodular forming with sever hyper atrophy of mucosal goblet cells with various degree of sloughing of intestine (Fig. 5).

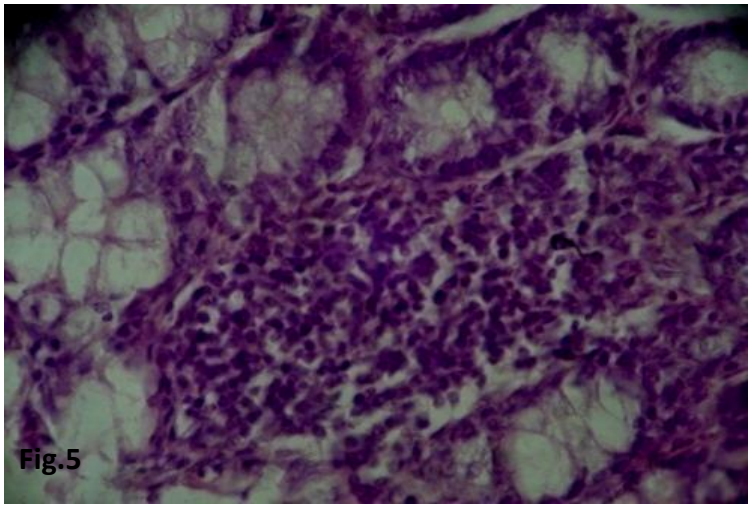

Figure 5: Photomicrograph of intestine of mouse infected with *S.mbandaka* at 4 week PI shows intense lymphatic aggregate with nodular appearance together with sever hyperatrophy of mucosal goblet cells. (H&E 40X).

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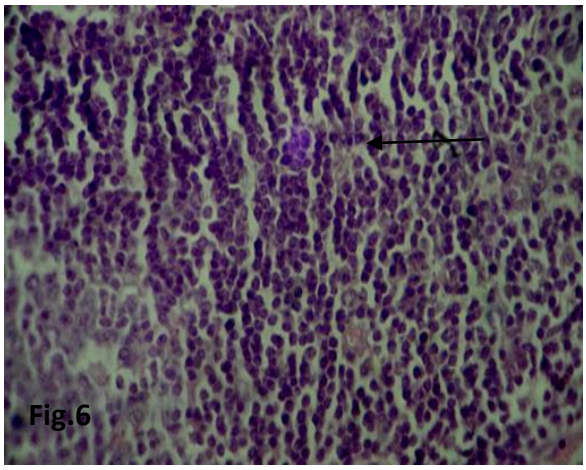
Histopathological examination of infective animals sacrificed after 6 weeks PI characterized by intense lymphoid hyperplasia in payer's patches of intestine(Fig. 6). The lung showed intense MNCs perivascular aggregate with congestion blood vesicles (Fig. 7).



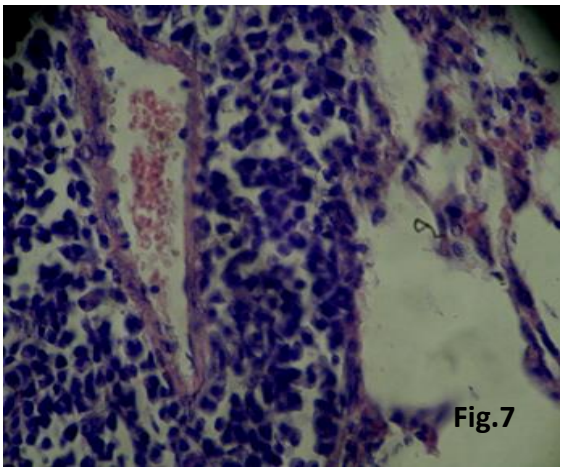


Figure 6: Photomicrograph of intestine of mouse infected with *S.mbandaka* at 6 week PI shows intense lymphoid hyperplasia in pyers patches ◄——(H&E 40X).

Figure 7: Photomicrograph of lung of mouse infected with *S.mbandaka* at 4 week PI shows intense MNCs perivascular aggregate with blood vessles congestion (H&E 40X).

The histopathological examination of infective animals sacrificed after 8 weeks PI showed no clear pathological changes in most organs except minimal desquamated mucosal layer with sub epithelia cellular infiltrate of intestine (Fig.8).

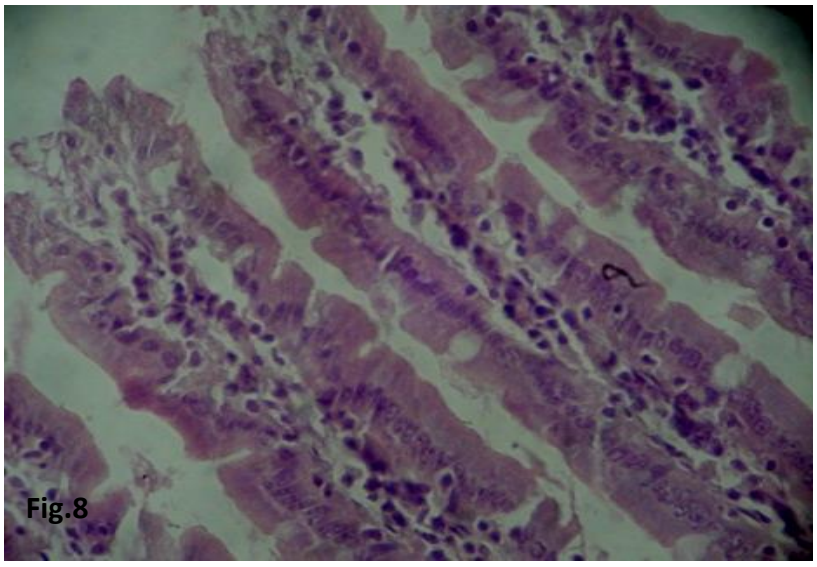


Figure 8: Photomicrograph of intestine of mouse infected with *S.mbandaka* at 8week PI shows minimal desquamated mucosal layerwith sub epithelia cellular infiltrate . (H&E 20X).

According to histopathological examination the lesions showed that *Salmonella mabandaka* which used in the current study can produce significant changes in the internal target organs of experimental infected mice mainly in intestine and this may be attributed to its primary multiplication in the lumen of intestine that causes changes in the composition of the lumen and enhance inflammation in the mucosa and L.p efficiently disseminate to another host, ensuring success for pathogen invasion (Bliska &Velden 2012).

Salmonella colonizes the Peyer's patches of the intestine and penetrates the gut barrier via M-cells from which it can disseminate to the local mesenteric lymph nodes and then to the spleen and liver, transported by phagocytic cells and when *Salmonella* invades the blood stream, it reaches different and distal target organs or tissues where it is able to multiply and cause more or less severe systemic focal infections (Rodriguez *et al*., 2006). The ability of DC to migrate throughout the body potentially facilitates the spread of *Salmonella* to various parts of the body ; While in the DC the *Salmonella* does not appear to replicate but remains viable, possibly in a small colony variant state with reduced metabolic activity and increased persistence (Tierrez and Garcia-del Portillo, 2005).

The macrophages or denderitic cells enter certain organ systems, the *Salmonella* can spread to adjacent cells and trigger apoptosis, which leads to increase pathology among the infected cells (Sheppard *et al*., 2003). Moreover the results also recorded various degree slough of intestinal mucosa as well as well as shortening of villi, and this was in consistence with observation by (Cousaemeni *et al*., 1982) who suggest the shortening and loss of microvilli is in accordance with decreased alkaline phosphatase activity, and this enzyme is located in the plasma membrane of the microvilli and is considered as a measurement of the digestive-absorptive surface. In addition Yousif and Al- Nageeb ,(2010) mentioned the ultrastructural changes in the ileum of mouse inoculated with infected dose of *S. hader* that killed after 72 hours post infection were similar to those described in the previous intervals. More evident damage of the ileum was observed after passage of 96 hours there were loss of some microvilli, marked dilatation and vacuolization of the endoplasmic reticulum with dispersion of microvilli and loss of the other mainly structures of the injured enterocytes due to presence many intracellular bacteria and after 120 hours post infection revealed hypertrophy of goblet cell, dilatation of endoplasmic reticulum, severe cytoplasmic vacuolization, thickening of the nuclear membrane and there was several *Salmonella* containing vacuoles.

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