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Pathology of internal organs after infection of mice experimentally with LD50 dose of *Salmonella mbandaka* through the oral route

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Abstract
The present was designed to study the histopathological variations of *Salmonella mbandaka* isolated from child on healthy animals first time in Iraq. Twenty BALB/c mice were used during the period from February to May 2017. Animals were separated into equal groups (10 mice/group) then drenched orally as the following: 1st group by lethal dose ($10^{9.5}$cells/ml) of *Salmonella mbandaka*, 2nd group with 0.5 ml PBS as control group. Post 24 hrs of infection observed 50% of animals in 1st group were dead and sacrificed lived mice after 3 days PI. The results revealed pathological changes on internal organs as: (intestine, lung, liver, spleen and kidney) manifested by sever sluphing and desquamation with cellular debris in their lumen, sever suppuration with congestion, extension destruction of liver parenchyma, sever hemorrhage of splenic parenchyma with atrophy of white pulp and sever congestion with hemosidern pigment deposition active hyperemia and congestion between renal tubules, respectively.

Keywords: salmonellosis, pathological changes, lethal dose (LD50) and mice

1. Introduction
Nontyphoidal *Salmonella* enterica is often associated with human *Salmonella* infection and causes gastroenteritis to systemic infections [1]. Some *Salmonella spp.* are zoonotic frequent causes of foodborne illness and the most common serotypes isolated in Alberta from contaminated poultry and poultry products were *Salmonella mbandaka*, *Salmonella Heidelberg* and *Salmonella kentucky* [2].There are more than 2600 serovars capable of infecting both human and animal and causing significant global morbidity and mortility [3]. *Salmonella typhimurium* causes a severe effect on ileum, spleen were distinguished by edematous villi with splenomegaly in BALB /c mice [4]. The study of pathogenesis of *S. heidelberg* infection in weanling pigs was conducted by Reed *et al*. [5] who noticed that ultra-structural examination of the *S. Heidelberg* infection of ileal loops at 2 hours post inoculation, revealed normal appearing, absorptive and crypt epithelium and despite the presence of large numbers of organisms in the lumen and overlying the microvillus border. The microvilli were covered by abounded of mucus, debris and cytoplasmic components of degenerated and disrupted luminal cells. At 4hours after inoculation, in addition to an abundance of mucus and bacteria in the lumen, there were many more extruded degenerating cells than at 2hours post infection; these cells usually contained numerous bacteria within phagosomes and were not readily identified because of marked degeneration. Then after 24 hours, epithelial cells covering markedly short villi which were low cuboidal, with irregularly shaped nuclei. Microvilli were less plentiful, small and irregularly shaped. Intracellular bacteria were demonstrated only in mononuclear cells of the lamina properia. In rabbits Panda *et al.*, [6] isolated *Salmonella typhimurium* from most internal organs that be collected tissues such as "gastrointestinal tract, spleen, liver, heart, lungs and kidneys" at necropsy finding. So, Shallal *et.al*, [7] studied the pathomorphological effects in mice after drenched orally by (10^7)cells/ml of *Salmonella mbandaka.* Moreover, gross pathological changes and clinical haven't presented. Therefore, Shallal *et.al*, [8] designed to study the bacteriological effect, clinically and macroscopically aspects of *Salmonella mbandaka*. This study aimed to record all pathological changes in the internal organs of mice infected with virulent Salmonella *mbandaka.*

2. Material and methods
2.1 Laboratory animals
This study was conducted from February to May 2017.

Twenty, adult healthy (BALB/c mice) of both gender, obtained from the National Center of Research and Drugs Monitor in Baghdad.
The mice were divided equally into two groups.

The first group
Were drenched orally with LD50 dose ($10^{9.5}$ cells / ml) organism of *S. mbandaka* strain estimated by Shalla [9].

The second group (control group)
Were inoculated (0.5) ml of PBS.
Then kept separately in different places of the experimental house. The viable accounts of the bacteria were made according to [10].

2.2 Bacterial strain
Salmonella mbandaka isolates from feverish child with sever diarrhea in AL-Kadhimiya Hospital of Iraq, and dehydrated. This bacteria was identified according to [11]. Finally the isolates confirmed in National Center of *Salmonella* in Baghdad /Ministry of public health.

2.3 Pathological study
Mice from each group were observed daily for clinical signs and mortality and sacrificed by neck dislocation after 3 days post infection. Organs (Intestine, liver, spleen, lung and kidney were removed in aseptic conditions and kept in ten percent(neutral buffered formalin) for 24 hrs. The routine of histological process was performed to obtain slides stained by "Haematoxylin and Eosin" for pathological evaluation [12].

Abbreviations: PI= post infection, CFU= colony forming unit, PBS= phosphate buffer saline.

2.4 Statistical analysis
The Statistical Analysis System – SAS (2012) program was used to effect of difference factors in study parameters.

3. Results
Bacterial isolation and Histopathological studies
Salmonella mbandaka was isolated from all the cultured samples of sacrificed infected mice. While control mice showed negative results. The histopathological examination of internal organs infected mice 3days post infection were characterized by sever sluphing and desquamation of intestinal mucosa with cellular debris in their lumen (fig 1). The lung histolesions showed sever suppuration with blood vesicles congestion (fig 2). Perivascular cellular infiltration in liver tissue associated with extension destruction of liver parenchyma as well as sever congestion and hemorrhage (fig 3).The pathological changes of spleen showed sever hemorrhage and congestion splenic parenchyma associated with lymphoid depletion of white pulp (fig 4).In another section of spleen showed white pulp atrophy with sever congestion with hemosidern pigment deposition (fig 5). The histopathological examination of kidney showed active hyperemia and congestion between renal tubules that showed cellular swelling and aggregate around blood vesicles (fig 6).

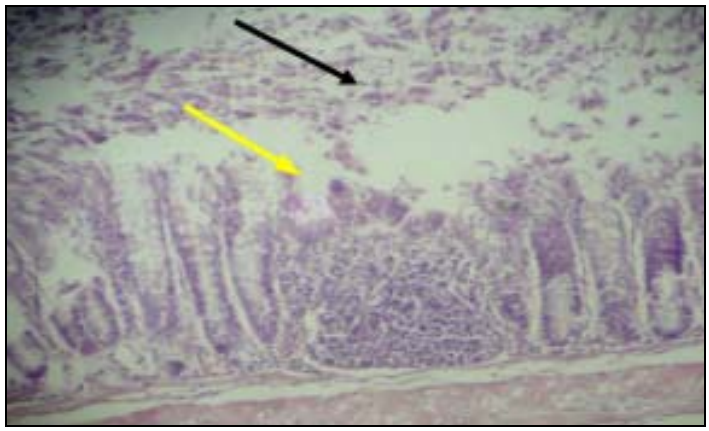

Fig 1: Intestine of a mouse orally infected with *S. mbandaka* showing sever sluphing and desquamation of intestinal mucosa (yellow arrow) with cellular debris in their lumen after 3 days of infection (black arrow). (H&E 40X)

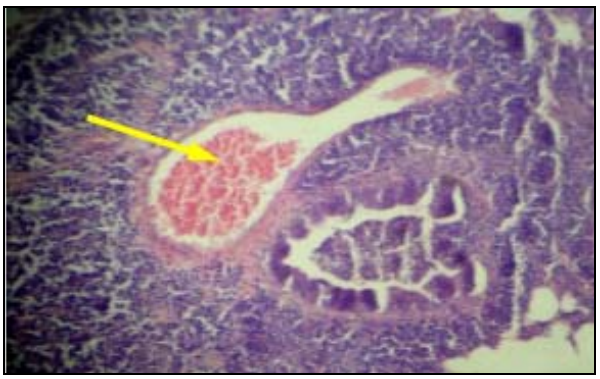

Fig 2: Lung of a mouse orally infected with *S. mbandaka* showing sever suppuration with blood vesicles congestion (yellow arrow). (H&E 40X)

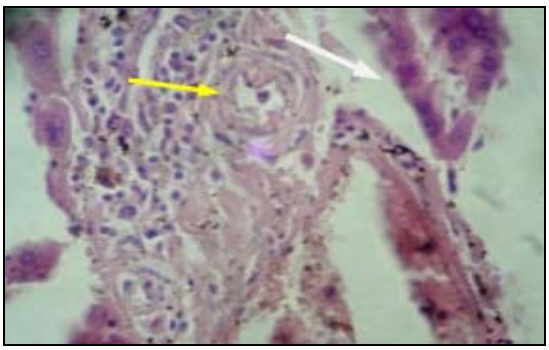

Fig 3: Liver of a mouse after 3 days infection with *S. mbandaka* shows perivascular cellular infiltration in liver tissue associated with extension (yellow arrow) and hemorrhage (white arrow). (H&E 40X).

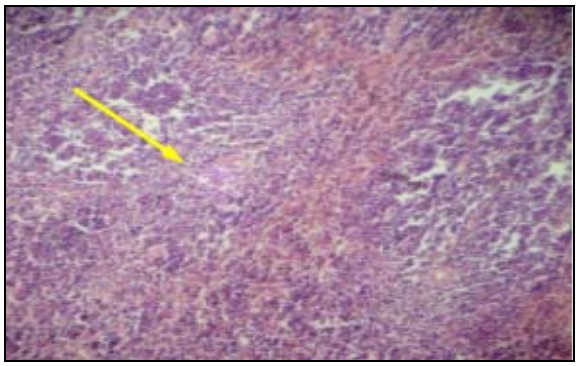

Fig 4: Spleen of a mouse after 3 days post infection of *S mbandaka* showing sever hemorrhage and congestion splenic parenchyma associated with lymphoid depletion of white pulp (yellow arrow). (H&E 40X).

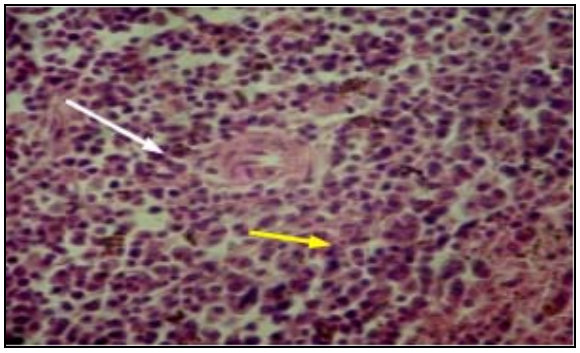

Fig 5: Spleen of a mouse after 3 days post infection of *S mbandaka.* showing white pulp atrophy with sever congestion (yellow arrow) with hemosidern pigment deposition (white arrow). (H&E 40X).

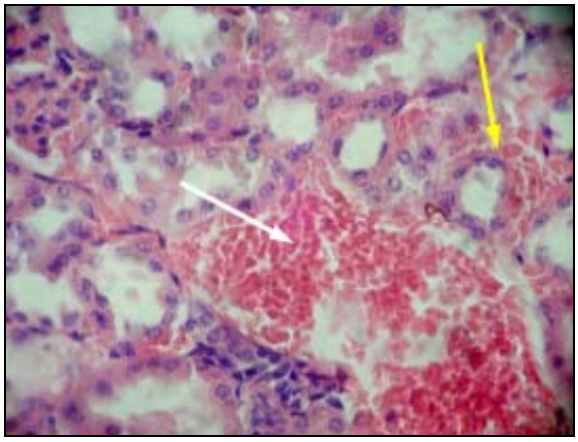

Fig 6: Kidney of a mouse after 3 days post infection of *S mbandaka.* showing active hyperemia and congestion between renal tubules (white arrow) that showed cellular swelling cellular aggregate around blood vessels (yellow arrow). (H&E 40X).

4. Discussion

Salmonella mabandaka revealed significant pathological 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, ensuring success for pathogen invasion in intestine and this evidence agreement with study Yousif and AL-Naqeeb [13] observed the detachment and disruption of microvilli, sever disruption and disorganization of the M-cell. Furthermore, Boyle *et al.* [14] record the early pathomorphological changes of small intestine in rats yet infected with *Salmonella spp.* were represented by the aggregation of cells were not contributory in the inflammation induction, nutrient absorption deficiency, accumulation of fluids and vasoconstriction showed. Likewise by Shallal *et al*, [7] referred the intestine of mouse infected orally with infective dose(1.3×10^7 cells/ml) of *S. mbandaka* at 2week post infection shows slight cellular infiltration with shortening of mucosal villi and slight cellular infiltration with sever hyperplasia mucosal gland of intestine. Moreover, Lalsiamthara and Lee, [15] confirmed the *Salmonella montevideo* in birds were mostly infecting the splenic parenchyma, cecum (intestinal epithelial) and minimally liver after oral infection without obvious illness appear on it. As well, Yass, [16] observed the pathological examination of microvilli in calves infected experimentally with *Salmonella typhimurium* by electron microscopically were noticed local derangement accompanied with slight swelling of the proximal end and presence of many bacteria adherent to the microvilli and became short and more effacement from epithelial cells in some location. The pathological changes after 3 days post infection reveals more ranged between white pulp atrophy with sever congestion with hemosidern pigment deposition to presence perivascular cellular infiltration associated with extension destruction of liver parenchyma as well as sever congestion and hemorrhage this evidence agreed with Nesterenko *et al*, [17] that mentioned the chronic infection in I/St mice infected with *Salmonella* was histological effects in spleen (splenomegaly), on the contrary A/Sn mice produced a higher level of *Salmonella* specific IgA forming cells in Peyer patches that leads to protection of A/Sn line during per oral infection. Zhu *et al*, [4] found that the infection of BALB/c mice with *Salmonella typhimurium* leads to increased levels of splenomegaly and dynamic changes in energy metabolism of the development of salmonellosis in mice. Moreover, the histopathological analyses revealed that *Salmonella gallinarum* caused fibrinoid necrosis in spleen and liver associated with infiltrates of macrophages, lymphocytes, heterophils and plasma cells [18]. Also previous observation by (Fantuzzi *et al*, and Sheppard *et al*,) [19, 20] they observed diffuse of small mixed inflammatory cells and infiltrate in hepatic parenchyma. On contrast to histopathological examination revealed by Lima-Filho *et al*, [21] that showed the necrosis of spleen and infiltrate inflammatory cells at nearly (10^4) cells /gram of organ at 28 days post infection; Whereas no bacteria were isolate from liver in the same stage. Fantuzzi *et al*, [19] reported pathological changes of spleen, liver, small intestine and mesenteric lymph nodes in mice infected orally (10^6)CFU of *S. typhimurium*. In the kidney of mice which drenched orally with (1.3×10^7) cells/ml of *S. mbandaka* at two weeks post infection 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 and marked granulomatous like reaction in the renal tissue seen mainly in periglomaruli accompanied with tubular necrosis with MNCs infiltration consist mainly of macrophages at six week [7].

5. Conclusion

From the result of this study, it can concluded salmonellosis present a real public health problem throughout the Iraq. And indicate the ability of *Salmonella mbandaka* to invade and replication in internal organs and induce disease.

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7. Reference

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