



## Research Article

### Study the Pathological Changes in the Intestine of Rabbits Infected Experimentally with *Salmonella typhimurium*

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#### ABSTRACT

The current study aimed to explore the histopathological changes in intestine of rabbits experimentally infected with *Salmonella typhimurium*. A highly virulent isolate of *S. typhimurium* obtained from Department of internal and preventive medicine/ College of Veterinary Medicine/University of Baghdad were previously diagnosed and confirmed by PCR. Infective dose of bacteria was prepared and given to animals at a dose of  $5 \times 10^9$  CFU.

The experimental study was conducted on 25 local rabbits of both genders aged between 2-4 months old were adapted for two weeks before starting the experiment. These rabbits divided randomly into five Groups, each group contains 5 rabbits, as follows: **Group 1:** these were used as negative control **Group 2:** these were used as infected group which drenched 5 ml suspension which have ( $5 \times 10^9$  CFU) of *Salmonella typhimurium*, **Group 3:** these were given a same dose of *Salmonella typhimurium* then treated with single dose of gentamicin alone at 0.05ml/kg (5mg/ml) orally after presence of signs, **Group 4:** these were given a same dose of *S. typhimurium* then treated with a single dose of Ca-EDTA alone at 40mg/kg orally after presence of signs, **Group 5:** these were drenched with the same dose of *S. typhimurium* then treated with combination of single dose of gentamicin at 0.05ml/kg (5mg/ml) orally and Ca-EDTA 40mg/kg orally after presence of signs.

The presented results of post mortem showed a congestion of intestine and filled with watery diarrhea. The results of histopathological examination of intestine revealed presence of different changes as infiltration of PMNs, destruction of crypts, villus atrophy and mucosal and submucosal blood vessel congestion in G2, G3, G4 pre and post treatment at different times, while the histological architecture in the G5 appeared near to normal with mild PMNs hyperplasia in mucosa at 48 hrs., while, at 96h it showed normal histological appearance.

**Key words:** Salmonella, Intestine, Rabbit, Histopathology

#### INTRODUCTION

Salmonellosis is a broad term applied to enteric infections caused by a group of gram-negative bacteria of the genus *Salmonella*, belonging to the family Enterobacteriaceae (Ojo and Adetosoye, 2009).

In domestic species animals, infections of *Salmonella* are considered as main cause of mortality and morbidity (Murray, 1996). Septicemia caused by gastroenteritis is considered as the way that manifestation of clinical infections caused by *Salmonella* organisms. It is critical to rapidly identify active infections within a herd or in patients at a veterinary hospital, because the organism can

be easily disseminated among patients through the fecal-oral route and result in environmental contamination (Newton-Clarke, 1995).

The study of pathogenesis of *S. heidelberg* infection in weanling pigs was conducted by (Reed et al., 1985) who noticed that ultrastructural 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 abundance of mucus, debris and cytoplasmic components of degenerated and disrupted luminal cells. At 4 hours

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after inoculation, in addition to an abundance of mucus and bacteria in the lumen, there were many more extruded degenerating cells than at 2 hours 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 propria. In rabbits Panda *et al.*, (2014) isolated *Salmonella typhimurium* from most internal organs that be collected tissues such as “gastrointestinal tract, spleen, liver, heart, lungs and kidneys” at necropsy finding. This study was aimed to explore the histopathological changes in intestine of rabbits experimentally infected with *Salmonella typhimurium*.

## MATERIALS AND METHODS

A highly virulent isolate of *S. typhimurium* obtained from Department of internal and preventive medicine/ College of Veterinary Medicine/University of Baghdad were previously diagnosed and confirmed by PCR as mentioned in previous study (Hasan *et al.*, 2018, Hasan *et al.*, 2018). Infective dose of bacteria was prepared as described by Miles and Misra, (1938) containing  $5 \times 10^9$  CFU as recommended by Habasha *et al.* (2009).

The experimental study was conducted on 25 local rabbits of both genders aged between 2-4 months old were adapted for two weeks before starting the experiment. These rabbits divided randomly into five groups as follows: **Group 1:** five rabbits were used as negative control which drenched orally by plastic syringe with normal saline at dose 5 ml, **Group 2:** five rabbits were used as infected group which drenched 5 ml suspension which have ( $5 \times 10^9$  CFU) of *Salmonella typhimurium*, **Group 3:** five rabbits were drenched 5 ml suspension having ( $5 \times 10^9$  CFU) of *Salmonella typhimurium* then treated with single dose of gentamicin (Vapco, Jordan) alone at 0.05ml/kg (5mg/ml) orally (according to manufacturer) after presence of signs (24hrs. post infection), **Group 4:** five rabbits were drenched 5 ml suspension containing ( $5 \times 10^9$ ) CFU of *S. typhimurium*

then treated with a single dose of Ca-EDTA (GLS company, USA) alone at 40mg/kg orally (according to manufacturer) after presence of signs (24hrs. post infection), **Group 5:** five rabbits were drenched 5 ml suspension containing ( $5 \times 10^9$ ) CFU of *S. typhimurium* then treated with combination of single dose of gentamicin at 0.05ml/kg (5mg/ml) orally and Ca-EDTA 40mg/kg orally after presence of signs (24hrs. post infection).

**Post mortem examination** of intestine for all groups at 48 and 96 hrs. after presence of signs and after treatment.

**Histopathological examination of intestine** at 48 and 96 hrs. after presence of signs and after treatment. The histopathology was performed according to (Luna and Lee, 1968).

## RESULTS

The current results of post mortem changes showed a congestion of intestine (Fig. 1) and filled with a watery diarrhea (Fig. 2). The histopathological examination of intestine revealed a normal and regular crypts, villi and low cellularity of stroma (Fig. 3) in control negative group, but the infected group at 48 hrs. showed sloughing of villi, necrotic mass (crypt abscess) of intestinal lumen and infiltration of inflammatory cells (Fig. 4), also, infected group at 96 hrs. characterized by destruction of crypts with villus atrophy and massive PMNs infiltration with dilated and congested blood vessels in submucosa and mucosa (Fig. 5).

The current results also showed severe infiltration of inflammatory cells and atrophied intestinal glands and blunting of the villi at 48hrs in gentamicin treated group (Fig. 6), while after 96hrs post treatment it showed severe perivascular cuffing of inflammatory cells with severe blood vessel congestion in all intestinal layers (Fig. 7).

The histopathological lesions of the intestine of group 4 showed severe inflammatory cell infiltrations with blood vessel congestion with edema in (sub) mucosa (Fig. 8).

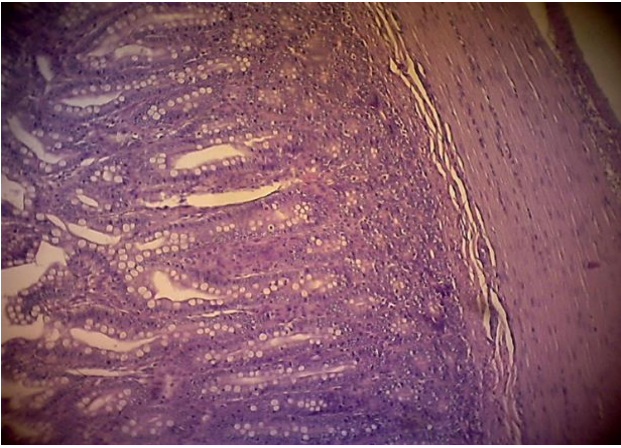
The group 5 showed a histological architecture near to normal appearance with mild PMNs hyperplasia in mucosa at 48 hrs. (Fig. 9), while, at 96h it showed normal histological appearance (Fig. 10).



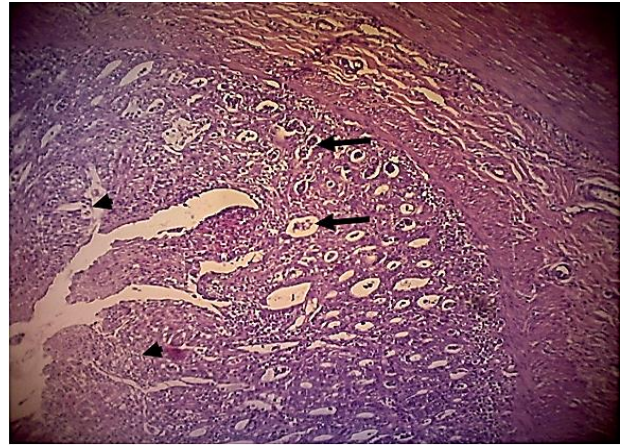
**Fig. 1:** The congestion of large intestine and filled with feces



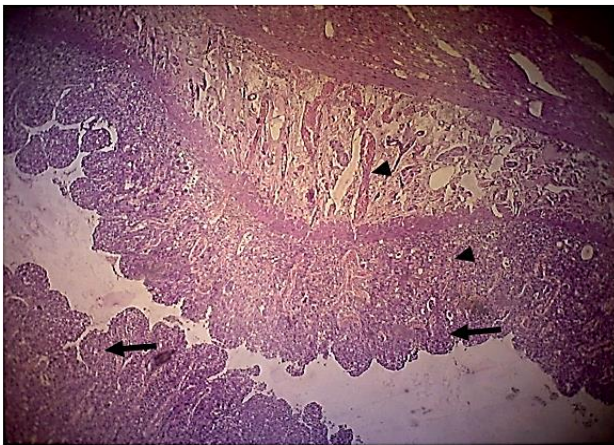
**Fig. 2:** Small intestine were flaccid and filled by yellow watery feces with mucus



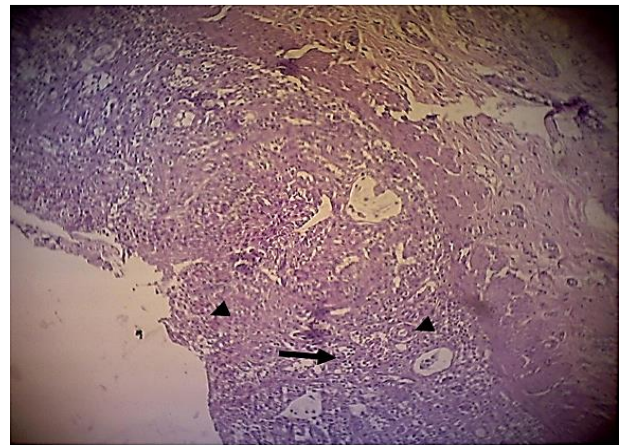
**Fig. 3:** Normal control. Show normal and regular crypts and villi and low cellularity of stroma, HE stain, (x4).



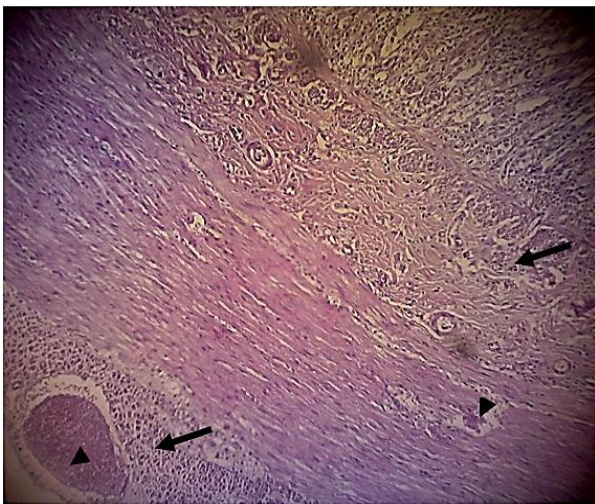
**Fig. 4:** Intestinal section of infected group at 48 h. shows sloughing of villi (arrow head), necrotic mass (crypt abscess) (arrow) of intestinal lumen and infiltration of inflammatory cells, HE stain, (x4)



**Fig. 5:** The histopathological lesions of the intestine of rabbits after infected group at 96 h characterized by destruction of crypts with villus atrophy (arrow) and massive PMNs infiltration with dilated and congested blood vessels (arrow head) in (sub)mucosa and mucosa, HE stain, (x4)



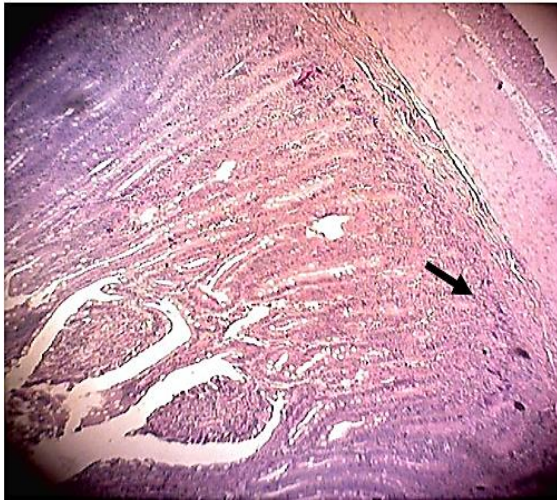
**Fig. 6:** Intestine section of group 3 shows severe infiltration of inflammatory cells (arrow) and atrophied of intestinal glands and blunting of the villi (arrowhead) at 48h, HE stain, (x4)



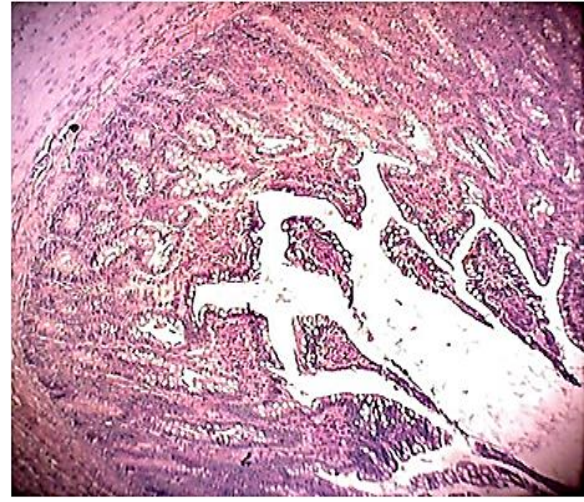
**Fig. 7:** Intestine section of group 3 shows severe perivascular cuffing of inflammatory cells (arrow) with severe blood vessel congestion (arrowhead) in all intestine layer at 96 h, HE stain, (x10)



**Fig. 8:** The histopathological lesions of the intestine of Rabbits after treatment with EDTA show severe inflammatory cell infiltrations (arrow) with blood vessel congestion (arrowhead) and wall with edema and in (sub) mucosa, HE stain, (x10)



**Fig. 9:** The intestine of treated group 5 show histology architecture near to normal appearance with mild PMNs hyperplasia in mucosa (arrow) at 48 h., HE stain, (x10)



**Fig. 10:** The intestine of treated group 5 at 96h show normal histology appearance (arrow), HE stain, (x10).

## DISCUSSION

The results of post mortem findings of salmonellosis in rabbits in the infected group resemble that recorded with (Lewis, 2006) who showed that *S. enteritidis* infection in rabbits is consistent with septicemia, congestion and petechiation of multiple organs, this could be the only noticed pathology in peracute conditions, also lymph nodes, myocardium, spleen, kidney and the liver presented necrotic foci surrounded via polymorphonuclear leucocytes could be visible in salmonellosis severe conditions. Also the results agree with (patton *et al.*, 2008) who reported that the highest prominent lesions located at necropsy in rabbits' *S. enteritidis* infection is found in intestine, lungs, mesenteric lymph nodes, spleen and liver. Hemorrhagic and ulcerative changes exists in intestine. Spleen and liver are commonly enlarged and consist of pale, pinpoint areas of necrosis.

The results of histopathology were agreeing the a result of Everest *et al.* (1999) who showed that lesions of the intestine after treatment with *S. typhimurium* orally characterized by hyperplasia of goblet cells, inflammatory cells in the lamina propria of atrophic villi. Also, Pathogenic *Salmonella* invades the mucosa of large and small intestine and create toxin and stimulate releasing proinflammatory cytokines and prompts severe inflammatory reaction and could cause destruction and ulceration of mucosa, the bacteria might disseminate from intestine to result in systemic infection (Monack and Falkow, 2004)

After treatment, the gentamycin treated group showed no response to treatment, also Ca- EDTA alone showed no any improving in health of infected rabbit, while the combination treatment (Gentamicin + Ca-EDTA) showed response to this therapy. These results were in agreement with result of Miller *et al.* (2004) suggested that EDTA therapy may reduce the oxidative stress injury and inflammation. The potentiation of gentamicin impact is very possible because of the direct bactericidal effect of EDTA against bacteria (Banin *et al.*, 2006). ROS are considered vital signaling molecules

which are highly significant in the development of inflammatory disorders (Mittal *et al.*, 2014), So that EDTA has the ability to lower the ROS levels and then decreasing inflammation (Fulgenzi *et al.*, 2014).

## Conclusions

*Salmonella typhimurium* infection causing intestinal lesion characterized by presence of blood vessel congestion with edema and infiltration of PMNs histopathologically.

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