Studying the pathogenesis of experimentally infected rabbits with *Salmonella typhimurium* Isolated from Milk and Milk Product

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Abstract

The current study aimed to explore the histopathological changes in organs of rabbits experimentally infected with *Salmonella typhimurium*, the second isolate; 39/288 (13.5%), from milk and milk products; collected in the period from August 2019 to April 2020. 18 mature local rabbits of 1-1.900 kg body weight, from both sex, were used. After 2 weeks of adaptation to the environment of the farm of Department of Veterinary Medicine, College of Veterinary Medicine, University of Diyala, the animals divided into three groups (6 each), those of 1st group left without exposure to *Salmonella typhimurium*, while those of 2nd group divided into two subgroups, 1st one received cortisone at a therapeutic dose 0.1mg intramuscular for two times with three days interval, the 2nd subgroup received cortisone for one time at the same dose, then rabbits of this group and 3rd group which did not receive cortisone exposed to bacteria prepared and given to animals at a dose rate of 2x 10⁹ CFU twice three days apart orally. The dependent parameters were clinical, hematological, and histopathological changes. The results revealed that exposure to *S. typhimurium* results in the appearance of inappetence, isolation, depression, death. The gross lesions and histological changes were more severe in those exposed to cortisone. The main gross and histopathological changes were in the heart, liver, lung, kidney, and gastrointestinal mucous membranes.

Keywords: pathogenesis; rabbits; *Sal. typhimurium*


Introduction

In domestic animals, infections of *Salmonella* are considered as the main cause of mortality and morbidity (Muarry, 1996). Septicemia caused by gastroenteritis is considered as the way that manifestation of clinical infections caused by Salmonella organisms. Infection with Salmonella may be a result of cattle and dairy products constitute an important reservoir for numerous serotypes of those pathogens (Huartado et al., 2017; Park et al., 2014; Pesciaroli et al., 2017; Sivaramalingam et al., 2013). The virulence of pathogenic *Salmonella* spp. is associated both with chromosomal and plasmid genes (Chaudhary et al., 2015), in the bacterial chromosome, there are large gene cassettes, called pathogenicity islands, (SPIs), which code nearly 60 genes responsible for specific interactions with the host organism (Chaudhary et al., 2015 and Lahiri et al., 2015).
Salmonella spp. infection cycle starts after the ingestion of microbes. Through the stomach, the bacteria reach the small intestine. The pathogenicity of Salmonella spp. depends on the serotype and the host's immunity, and its virulence is determined by the factors: adherence to host's cells; invasion and replication inside host's cells; polysaccharide coating and production of toxins. (Figueiredo et al., 2015 and Ingram et al., 2017). Salmonellosis is caused by all nontyphoid serotypes of the Salmonella genus (excluding S. Typhi and S. paratyphi A, B, and C), isolated both from humans and animals, including livestock (Kurtz et al., 2017). Serotypes S. typhimurium, S. enteritidis, S. Newport, and S. Heidelberg are most often responsible for food poisoning., but S. cholerasuis and S. Dublin also cause diarrheic diseases (Andino and Hanning, 2015; Kurtz et al., 2017). Salmonella spp. is increasingly present in cattle, both bred for meat and milk (Oueslati et al., 2016). Cattle may be asymptomatic carriers of the pathogenic bacteria (the bacteria may remain in their gastrointestinal tract for a period ranging between a few months and a year), but there may also be symptoms of infection present, including diarrhea, fever lasting for up to 7 days, anorexia, dehydration, reduced milk production, miscarriages, or the presence of toxins in the blood (Hoelzer et al., 2011; Cummings et al., 2009). Mortality associated with Salmonella spp. infections are high in calves but still not very common, just like miscarriages. The aims of the study were study the histopathological changes in organs of rabbits experimentally infected with Salmonella typhimurium

Material and methods

Eighteen mature local rabbits of 1-1.900 kg body weight, from both sex, were used. After 2 weeks of adaptation to the environment of the farm of Department of Veterinary Medicine, College of Veterinary Medicine, University of Diyala, the animals divided into three groups (6 each), those of 1st group left without exposure to Salmonella typhimurium, while those of 2nd group divided into two subgroups, 1st one received cortisone at a therapeutic dose 0.1 mg intramuscular for two times with three days interval, the 2nd subgroup received cortisone for one time at the same dose, then rabbits of this group and 3rd group which did not receive cortisone exposed to bacteria prepared and given to animals at a dose rate of 2x 10^9 CFU twice three days apart orally. The depended on parameters, monitoring the animal to record any changes in behavior, appetite, feces or any signs, together with Clinical parameters (Bodyweight, Body temperature, heart rates, and respiratory rates), were done according to (Radostitis, et al., 2007); Hematological, included (Hb concentration, and HCT%; Total and Differential leucocytes counts) according to (Coles, 1998) were done at starting of experiment, 6th day of the experiment, and at a time of sacrifice of animals, with the help of ether as an anesthetic agent. Each animal was examined grossly to record the gross changes in animal viscera. With samples from gastro-intestinal, heart, lungs, kidney, testes, liver for histopathological examination, the histopathological (hematoxylin - Eosin stain) was performed according to (Luna and Lee, 1968).

Statistical analysis

Statistical analysis of data was performed using ANOVA, unpaired t-test, and the lowest significant differences (LSD). All experimental data are presented as Mean ± S.E. The results were considered significant if P < 0.05. (Steel et al., 2007)
Results

From a total 186 samples; represent 76 samples raw milk; 35 milk products and 75 samples from workers in shops of milk products: 288 isolates were isolated. The highest numbers of isolates was *Staphylococcus* 58/288 (20.1%); *Salmonella* 39/288 (13.5%); *Lactobacillus* 35/288 (12.2%); *E. coli* 30/288 (10.4%); *Pseudomonas* 30/288 (10.4%); *Klebsiella* 29/288 (10.1%); *Enterobacter* 24/288 (8.3%); *Proteus* 17/288 (5.9%); *Citrobacter* 17/288 (5.9%); *Streptococcus* 5/288 (1.7%); *Listeria* 4/288 (1.4%) (Table-1-)

![Table 1: Total numbers of isolates in current study](image)

### Table 1- Total numbers of isolates in current study

<table>
<thead>
<tr>
<th>Origin</th>
<th>No.</th>
<th>Isolates sp.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worker</td>
<td>75</td>
<td>17 19 27 5 0 4 4 0 4</td>
<td>85</td>
</tr>
<tr>
<td>Raw</td>
<td>76</td>
<td>8 5 24 15 27 21 17 12 12 0 0</td>
<td>141</td>
</tr>
<tr>
<td>Prod.</td>
<td>35</td>
<td>4 6 7 10 12 14 3 1 5</td>
<td>62</td>
</tr>
<tr>
<td>Total</td>
<td>186</td>
<td>29 30 58 30 39 35 24 17 17 5 4</td>
<td>288</td>
</tr>
</tbody>
</table>

Clinical changes

The animals exposed to *Salmonella typhimurium* exhibit signs of depression, anorexia, isolated, soft feces. Heart rates increased in GIII. Respiratory rates significantly increased in GII and GIII. Body temperature and body weight no significant changes. (Table -2-)

![Table 2: Heart rates /min, respiratory rate/min, Body temperature °C, and Bodyweight of rabbits used in study](image)

### Table 2- heart rates /min, respiratory rate/min, Body temperature °C, and Bodyweight of rabbits used in study.

<table>
<thead>
<tr>
<th>parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>H.R./min</td>
<td>158.7 ± 7.74a</td>
<td>157.5 ± 8.54a</td>
<td>151.5 ± 5.0 6a</td>
</tr>
<tr>
<td>R.R./min.</td>
<td>129.5 ± 3.59a</td>
<td>128.0 ± 6.38a</td>
<td>128.5 ± 12.</td>
</tr>
</tbody>
</table>
Hematological changes

Total leucocytes counts and Lymphocytes % increases in those of GII, and GIII. Others showed no significant changes (Table -3-).

Table -3- hematological pictures of rabbits used in the study

<table>
<thead>
<tr>
<th>parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>11.1±</td>
<td>11.28±</td>
<td>11.38±</td>
</tr>
<tr>
<td></td>
<td>0.35a</td>
<td>0.45a</td>
<td>0.51a</td>
</tr>
<tr>
<td>PCV%</td>
<td>34.25±</td>
<td>37.25±</td>
<td>36.0±</td>
</tr>
<tr>
<td></td>
<td>2.10a</td>
<td>1.93a</td>
<td>3.24a</td>
</tr>
<tr>
<td></td>
<td>4.68a</td>
<td>0.70a</td>
<td>0.50a</td>
</tr>
<tr>
<td>Lymph.%</td>
<td>46.5±</td>
<td>45.25±</td>
<td>46.75±</td>
</tr>
<tr>
<td></td>
<td>3.80a</td>
<td>8.0a</td>
<td>5.0a</td>
</tr>
<tr>
<td>Neutrophil.%</td>
<td>47.75±</td>
<td>50.25±</td>
<td>46.80±</td>
</tr>
<tr>
<td></td>
<td>7.96a</td>
<td>6.05a</td>
<td>5.05a</td>
</tr>
<tr>
<td>Esinoph.%</td>
<td>2.25±</td>
<td>2.5±</td>
<td>2.85±</td>
</tr>
<tr>
<td></td>
<td>0.5a</td>
<td>0.19a</td>
<td>0.29a</td>
</tr>
<tr>
<td>Basophil.%</td>
<td>0.75±</td>
<td>0.5±</td>
<td>0.85±</td>
</tr>
<tr>
<td></td>
<td>0.25a</td>
<td>0.10a</td>
<td>0.15a</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2.8±</td>
<td>2.0±</td>
<td>2.65±</td>
</tr>
<tr>
<td></td>
<td>0.15a</td>
<td>0.10a</td>
<td>0.2a</td>
</tr>
</tbody>
</table>

Values are M±S.E., a, b significantly differ at P<0.05


Values are M±S.E., a, b significantly differ at P<0.05

Postmortem findings

Main gross lesions were in the liver, lung, heart, kidneys, and gastrointestinal tract. Severe congestions, with enlargement of viscera (Picture1-)

Histopathological changes

Animals, not received cortisone, exposed to *Salmonella typhimurium* at a dose rate of 2x10⁹ / ml, two ml, orally twice times, three days apart. The main histopathological changes were the Stomach showed areas of fibrosis and proliferation of mononuclear cells (neutrophils). Lung showed edematous fluid with erythrocytes Expansion and destruction of alveoli and thickening of some alveolar wall. The kidney showed focal intertubular MNCs Infiltration and many vacuoles. Figures (1-3).
Animals sacrificed, exposed to one dose of 0.1 mg hydrocortisone I.M. and exposed to $2 \times 10^9$ Sal. / ml, 2 ml, orally twice times, three days apart. The main histopathological changes were, Small intestine showed mucosal erosion & hyperplasia of peyer's patchy in the submucosa. The stomach showed hyperplasia of the mucosal gland and oozing of blood in the mucosal layer, this gives rise to hemorrhagic gastritis. Lung showed emphysema which characterized by highly alveolar wall distraction. Kidney showed proliferation of multinuclear cells (mesengeal cells), and severe vacuolar degeneration of occluded tubules. Figures 4-7).

Figure (3): Kidney showed focal intertubular MNCs Infiltration (→) and many vacuoles (←). (H&E stain 40X).

Figure (4): Small intestine showed mucosal erosion (arrow) & hyperplasia of peyer's patchy in the submucosa (H&E stain 40X).

Figure (5): Stomach showed hyperplasia of the mucosal gland (←) and oozing of blood in the mucosal layer (→), this gives rise to hemorrhagic gastritis, (H&E stain 40X).
Animals died, received hydrocortisone 0.1 mg I.M./ twice times three days apart, and exposed to Sal at $2 \times 10^9$ / ml, 2ml orally, twice time, three days apart. The main histopathological changes were, Small intestine showed thread of mucus from the goblet cells, vacuolar degeneration of mucosal glands in the submucosa of the small intestine, and hemorrhage. Lung showed distraction of alveolar wall and thickening of the alveolar wall in some areas (interstitial pneumonia) due to the proliferation of inflammatory cells (neutrophils) with a few mononuclear cells. Heart showed inflammatory cells in Interstitial and infiltration of mononuclear cells in the pericardium. Figures (8-10):
Figure (8): Small intestine showed thread of mucus from the goblet cells (↓), vacuolar degeneration of mucosal glands in the submucosa of the small intestine (↑) and hemorrhage (↓). (H&E stain 40X).

Figure (9): Lung showed the distraction of alveolar wall (↓) and thickening of the alveolar wall in some area (interstitial pneumonia) due to the proliferation of inflammatory cells (neutrophils) with a few mononuclear cells (↓). (H&E stain 40X).
Figure (10): Heart showed inflammatory cells in Interstitial (→) and infiltration of mononuclear cells in the pericardium (←), (H&E stain 40X).

Animals died, received hydrocortisone 0.1 mg I.M. one time, and expose to Sal 2x10^9 /ml, 2ml, orally twice, three days apart, the main histopathological changes were, Stomach showed desquamation of mucosal surface of the stomach with bleeding different area in the mucosal and submucosal layers. The kidney showed hemorrhagic area, the proliferation of mononuclear cells, and vacuolation, this case refers to hemorrhagic nephritis. Heart showed inflammatory cells in the interstitial and presence of vacuole. Figures (11-13):

Figure (11): The stomach showed desquamation of the mucosal surface of the stomach (←) with bleeding different areas in the mucosal and submucosal layers (←), (H&E stain 40X).

Figure (12): Kidney showed hemorrhagic area (←), the proliferation of mononuclear cells (←), and vacuolation (←), this case refer to hemorrhagic nephritis), (H&E stain 40X).

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In the current study, the animals exposed to *Salmonella typhimurium* exhibit signs of depression, anorexia, isolated, soft feces, diarrhea. Heart rates increased in GIII. Respiratory rates significantly increased in GII and GIII. Body temperature and body weight no significant changes. Total leucocytes counts and lymphocytes % increased as a result of exposure.

Typical symptoms of infection with nontyphoid serotypes of Salmonella spp. are stomach ache and diarrhea, but other symptoms include vomiting, nausea, fever, shivers, muscular or articular pain, cramps and loss of appetite (Hung *et al.*, 2017; Antillon *et al.*, 2017; Jarvis *et al.*, 2016; Hald, 2013). After the disappearance of symptoms, Salmonella may still reside in the intestine of an adult for 4 weeks, and in children for up to 7 weeks.

Salmonella is the etiological agent of both human and animal salmonellosis, a very common and widely spread enteric disease. It is a significant cause of acute and chronic diarrhea and death in numerous animal species and in human beings (McGavin *et al.*, 2001).

The gross and histological changes in the current study were mainly in the lung, heart, liver, kidney, gastrointestinal tract.

The steps in the pathogenesis of Salmonellosis may be broadly divided into at least three categories, (a) ability to invade the gastrointestinal mucosa; (b) ability to cause diarrhea; and(c) the ability to disseminated from the intestine and multiply and survive within the reticuloendothelial system.

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 findings. S.
typhimurium isolated from milk and milk products and workers previously diagnosed and confirmed by cultural, biochemical, and Vitek 2 (Hasan et al., 2018). A modified Infective dose of bacteria was prepared principally as described by Miles and Misra(1938), containing 2X10⁹CFU twice, three days apart orally as recommended by Habasha et al., 2009.

Pathogens enter the organism with water or foods infected with fecal microbiota. (Andino and Hanning,2015; Kurtz et al., 2017; Wiedemann et al., 2015). The infective dose ranges between 10⁶ and 10⁸ cells, but in some people, even the dose of 10 cells may lead to the development of salmonellosis (Antunes et al., 2016 and Jarvis et al., 2016). The incubation of the disease lasts for 6-72 h, depending on the infective dose and the host's condition. In the majority of cases, the infection lasts no longer than 7 days (Fabrega and Vila, 2013; Jiang et al., 2015 Jarvis et al., 2016).

The post mortem changes showed congestion of intestine and filled with watery diarrhea (Hussein, et al., 2019).

The histopathological examination of intestine revealed normal and regular crypts, villi, and low cellularity of stroma 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, also, the infected group at 96 hrs. characterized by the destruction of crypts with villus atrophy and massive PMNs infiltration with dilated and congested blood vessels in submucosa and mucosa (Hussein, et al., 2019).

The results of post mortem findings of Salmonellosis in rabbits in the infected group 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. (Lewis, 2006).

Patton et al., (2008) reported that the highest prominent lesions located at necropsy in rabbit's S. enteritidis infection are found in the intestine, lungs, mesenteric lymph nodes, spleen, and liver. Hemorrhagic and Ulcerative changes exist in the intestine. Spleen and liver are commonly enlarged and consist of pale, pinpoint areas of necrosis.

Everest et al., (1999). Showed that lesions of the intestine after treatment with S. typhimurium orally characterized by hyperplasias of goblet cells, inflammatory cells in the lamina propria of atrophic villi. Also, pathogenic Salmonella invades the mucosa of the 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 be disseminated from the intestine to result in systemic infection (Monack and Falkow, 2004).

The main gross lesion and histopathological changes were in the gastrointestinal tract. S. typhimurium infection is characterized by inflammation at the site of bacterial entry, typically the Peyer's patches (Carter and Collins, 1975). After Salmonella penetrates the epithelial barrier, it preferentially infects phagocytes within the lamina properia. In salmonella gastroenteritis, the infection is usually self-limiting and does not proceed beyond the lamina properia. In host-adapted salmonellosis, the salmonella -0 infected phagocytes

gain access to the lymphatics and bloodstream permitting the bacteria to spread to the liver and the spleen (Vasquez et al., 1999).

Conclusions

Salmonella typhimurium infection-causing intestinal lesion characterized by the presence of blood vessels congestion with edema and infiltration of PMNs

References


