Experimental Infection Of Salmonella pullorum To Study Immunological And Histopathological Changes Of Broiler

Tariq khalil Abed and Balqees Hassan Ali*

* Department of Pathology and Poultry Diseases, College of Veterinary Medicine, university of Baghdad

Abstract: Salmonella pullorum were isolated from the broiler chicken samples Rose. 12 isolates were identified from a total of 69 samples and identified by conventional cultural, motility and morphological characteristics. And was confirmed by PCR and then prepare a vaccine using the method of freezing and thawing repeated and this method has a clear effect on the growth of bacteria. The second step was to give the bacteria as a vaccine for a total of 30 chicks at the age of 7 days. The second dose was repeated at 14 days, the histological changes of the cecum and liver were observed at 20 days, and the immunological tests DTH, PHA were performed after collecting serum samples at the age of 24 days. Both groups were given a 0.5 ml dose of weakened bacteria and observed gross and histological changes of the internal organs of the chickens. Gross and histological changes were recorded in the chicks before and after the vaccine was administered. It has been concluded that the method of freezing and thawing has a clear effect as a vaccine.

Keywords: Salmonella pullorum, Immunological, Histopathological, and Broiler

I. Introduction

Salmonella enterica S. enterica include more than 2500 Serovars source the genus Salmonella, which is the most common from a species Salmonella, and is responsible for various diseases, many of these species have significant roles in the medical and veterinary fields, approximately 99.5% of the isolated species belong to S.enterica, which causes acute intestinal inflammation and rather than systemic diseases Grammatto et al., 2013. Pullorum disease PD and Fowl typhoid FT, are two diseases specific for avian, these diseases cause significant economic losses around the world. S. Pullorum causes PD infect young birds is an acute systemic. S. gallinarum causes FT effects at adult birds, and all ages may be susceptible to infection is an acute or chronic Barrow and Freitas , 2011. Salmonella transmission vertical from the mother to the chicks via egg, but remain the big problem is the horizontal transmission through carriage, feed, and vectors including humans, rodents, and insects Foley et al., 2008; Wales et al., 2013. Salmonella like many pathogens transmission by direct ingestion, also possible transmission through the inhalation Lopez et al., 2012. For the prevention and control of Salmonella can be used a vaccine, where he was important in the production of meat and eggs free of salmonella, both Live attenuated and killed vaccines with broad impact to reduce mortality and shedding pathogen to environment Priyantha 2009; Hossain 2011.

During the infection of Salmonella and after the arrival of these pathogens to the intestine begins the body with the innate immunity, which is considered the first line of defense, including epithelial layers, macrophages, complement, dendritic cells, coagulation cascade, cells act as natural killer and coagulation cascade, another function to these cells retain for intestinal homeostasis through integral pathogen signals Peterson and Artis ,2014.

II. Material And Methods

Field Study: Of the total 69 samples collected from broiler chickens, which had signs of respiratory and diarrhea, in the province of Salah al-Din, and was sent to the laboratory of the microbiology of Veterinary Medicine, the University of Tikrit for the purpose of examination and isolation of Salmonella pullorum.

Isolation and Identification of Salmonella spp. Procedure : The study was conducted using traditional methods of detection and diagnosis of Salmonella, following the standard guidelines from OIE, 2012 sample culture on Selenite F broth and incubated for 24 hours at 37°C. Then transfer a drop of broth using the Loop and subculture on Salmonella-Shigella agar and XLD for the purpose of testing their fermentation or non-fermentation of lactose sugar, and production of hydrogen sulfide gas H2S, incubated at 37 °C for 24 hours Menghistu et al., 2011. Further confirmations have been made on colonies suspected of Salmonella by conventional biochemical test.

Biochemical test: Biochemical diagnosis was based on the methods developed by Quinn et al., 2004, Where one or more pure colonies of isolated bacteria were identified, culture the bacterial colony were subjected to the slant
Triple Sugar Iron (TSI) agar, sugar Carbohydrate fermentation test, citrate utilization, urease reaction, indole reaction, and sulfide- indole- motility (SIM) Test.

**Half Lethal Dose (LD50):** The bacteria was growth on nutrient broth at 37 °C for 24 h, and in the centrifuge with 3000 cycles/min for 15 minutes were concentrated. By PBS three times, for five minutes each time were washed and discard the liquid, according to the following Reed and Mueneh.

**Freezing And Thawing:** This method was used according to Calcott and MacLeod, through freezing the bacteria, then frozen slowly 1 -2 °C/min to at least -70 °C before melting at a slow rate.

**Experimental chicks:**
The chicks that were used in the experiment were divided into two groups, the first of which consisted of 30 chick - a one- day Rose type called the experimental group and the second called control group, under similar conditions, fed on pellets and drinking water free of additives.

**First dose:** All chicks (30 chicks) were given 0.1 ml (2 x 10^7 cfu/ml) of the live attenuated bacteria at the age of 7 days.

**Second dose:** At the age of 14 days, all chicks received 0.1 ml 2 x 10^-7 cfu/ml of live attenuated Salmonella.

**Challenge dose:** At 28 days of age, all birds in two groups n = 15 were orally challenge with 0.5 ml 2 x 10^-7 cfu/ml of suspension containing Salmonella strain.

**Gross lesion:** Gross lesion was determined for each of the internal organs in two group vaccinated and control, and then show the change lesions in 28 day.

**Immunological tests:**
**Delayed-type Hypersensitivity test (DTH):** On day 20 post vaccination, 0.1 ml of S. pullorum were injected in the right wing, the left wing as control injected with 0.1 ml sterile PBS, at 24 and 72 hours right after injection the expansion diameter was measured. The results were expressed as the mean increase in the sensitivity area at periodic intervals after injection.

**Passive haemagglutination (PHA) test:** PHA test was used to determine the antibody titers in chickens vaccination by Salmonella pullorum. In 17 days after post vaccination on age 24 day, sera collected from the vaccinated group and control group stored at freezing until use. This test was conducted according to Herbert, 1987 methods.

**Sample collected:**
After S. Pullorum vaccination on day 7 day 0 of vaccine, and 2nd vaccine on 14 day day 7 of vaccine, 2 birds from two group were euthanatized at 20 days from age 13 day post vaccination. By cervical dislocation for gross and histopathological studies, the tissue samples of the liver and intestines cecum, were sent to the laboratory and are subject to formalin 10% for tissue samples. On day 28 21 post vaccination challenge dose 0.5 ml/bird and after two day, histopathological studies.

**III. Result**
In this study, a total of 12 57.1% S. Gallinarum / pullorum of 69 samples were identified as Salmonella sp. This was done through the use of cultural techniques and biochemical tests. Results obtained from the cultural, morphological and motility test of Salmonella isolates sp.

**PCR Result:** Salmonella isolates have been confirmed using the target genes specific to the A gene, using self-styling primers, 150bp for S. pullorum and 285bp for Salmonella sp.

**Determination of LD50:** Dilution 10^-5 showed the death of half the number of chicks when experimenting with Salmonella pullorum, which contains 200 cells / ml of a bacterial number. Thus, the LD50 lethal dose of 2 x 10^-7 cells/ml, as shown in table no

**Result Freezing and Thawing:** Salmonellapullorum was culture before freezing, with 250 cells at 1 mL after culture on stored agar at 37 °C / 24 h and 250 cells / ml. After thawing was 200 cells per 1 ml.

**Table 1:** Results of cultural, motility and morphological characteristics of the isolates of Salmonella spp.

<table>
<thead>
<tr>
<th>SS agar</th>
<th>XLD agar</th>
<th>Staining characteristics</th>
<th>TSI agar</th>
<th>Motality</th>
</tr>
</thead>
<tbody>
<tr>
<td>small, smooth</td>
<td>Colonies appear smooth with black center</td>
<td>Pink short rod, gram negative arranged in single or pairs</td>
<td>Black color colonies against a yellow background</td>
<td>Ve S. pullorum, S. gallinarum</td>
</tr>
</tbody>
</table>

DOI: 10.9790/2380-1012010109 www.iosrjournals.org 2 | Page
Experimental Infection Of Salmonella pullorum To Study Immunological And Histopathological..

Table 2: shows the number of death and alive for each dilution and bacterial account of this dilution.

<table>
<thead>
<tr>
<th>No.</th>
<th>Total sample sent to lab.</th>
<th>Broiler</th>
<th>Layer</th>
<th>positive</th>
<th>Negative</th>
<th>Positive%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>13</td>
<td>0</td>
<td>5</td>
<td>8</td>
<td>23.8%</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>18</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>9.6%</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>11</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>9.5%</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>19%</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>9.5%</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>60</td>
<td>9</td>
<td>21</td>
<td>48</td>
<td>100</td>
</tr>
</tbody>
</table>

**Gross Lesion results:** Gross lesions were observed, in the vaccinated group post challenge, after challenge 0.5 ml with *S. pullorum* recorded and photographed during necropsy, all the internal organs were normal: liver, intestine, heart, bile and kidney. The other organs no clear lesion observed. In control group gross changes were observed enlarged in the liver, with green-yellowish color, and friable, not in all cases figure 1. Kidney was enlarged, mild congested in intestine figure 2.

**Histopathological changes:** The results of the experimental infection in the chicken showed the following pathological changes in the organs cecum and liver, which were sampled at the age of 20 days, and two days after the challenge dose was given as follows:

In the cecum at 20 day found, the surface of the intestinal mucosa was covered by a simple columnar epithelium, and there was a degeneration of epithelial cells and a dissociation of the mass in the intestinal lumen, the goblet cells also hyperplasia. The inflammatory cells were invaded the epithelial cells at the mucosa. The lamina propria was highly engorged with inflammatory cells. Figure 3 show these effect. In between the bundles of smooth muscles fibers of tunica muscular showing lymphocytic infiltration. The blood vessels in the tunica serosa were congested with blood, with infiltration by inflammatory cells in the serosa fig.4.

**Figure 1:** A: Normal liver and intestine of vaccinated chicks at 28 day age. B: Normal internal organ kidney, heart and lung.
Experimental Infection Of Salmonella pullorum To Study Immunological AndHistopathological...

Figure 2: A: Mild hemorrhagic enteritis in control group at age 28 day after challenge with 0.5ml virulent SP. B: Normal intestine in vaccinated chicks reseved 0.5ml virulent SP.

Figure 3: Cecum of chicks at 20 day, show degenerated epithelial cells appear goblet cell hyperplasia and extensive inflammatory cell. H & EX 400.

Fig. 4: of the cecum of chicks 20 day vaccinated blood vessels congested, lymphocytic infiltration and serosa infiltration of inflammatory cells. H & E X 400.

After 28 days of age and two days after the dose challenge the following changes are observed:
- The mucosal villi were numerous, covered by simple columnar epithelium. Certain number of these cells were degenerated and invaded by inflammatory cells. These were masses of epithelial cells and mucus located in the lumen of intestine. As the figure 5.
Experimental Infection Of *Salmonella pullorum* To Study Immunological And Histopathological

In the liver at 20 day found. The parenchyma of the liver was containing a masses of the liver cells some of these cells are lost its nuclei, there was an atrophy of certain number of liver cell. The sinusoid were wide and highly extensive in between the masses of liver cells, these sinusoid appeared occupied by inflammatory cells with Kupffer cells. As the figure 6. Lymphocytic aggregation was noted around the central vein, which had congestion of blood figure 7.

**Figure 6:** liver at 20 day: appear liver cells atrophy nucleated inflammatory cells with kupffer cells, sinusoid wide and extensive between liver cell H & EX 200.

**Fig.7:** liver at 20 day of age, showing Lymphocytic aggregation around central vein . H & E X 200.
After 28 days of age and two days after the dose challenge the following changes in liver are observed: -The interstitial C-T was containing inflammatory cells, Kupffer cells loss nuclei. The capsular of the liver was containing foci of inflammatory cells **fig. 8.**

![Figure 8](image)

**Figure 8:** liver of vaccinated chicks at 28 day, observed Kupffer cells loss nuclei inflammatory cells in capsular and parenchyma H & E X 200.

The control groups after challenge with 0.5 ml *S. pullorum* at $2 \times 10^7$ cfu/ml in age 28 day, the cecum show degeneration the goblet cell, and sloughing in the lumen intestine inflammatory cell in epithelial cell of the mucosa **fig.9.**

![Figure 9](image)

**Figure 9:** cecum of control chicks after challenge 0.5ml of *SP* observed degeneration the goblet cell sloughing goblet cell in the lumen H & E X 200.

The parenchyma of the liver was containing a masses of many individual the liver cells some of these cells are lost its nuclei, the sinusoids were severely congested with blood. With presence of Kupffer cells in side these sinusoids **fig.10.**
Experimental Infection Of Salmonella pullorum To Study Immunological And Histopathological ...
Fig. 11; DTH reaction of skin increased thickness and scab formation after 48 h injection s. c. 0.1 ml from S. pullorum.

IV. Discussion

Jacirol Islam et al. 2016, and others found that the rate of Salmonella pullorum was 47.73% n = 21 which is more than what was achieved in our study which was 57.1% n= 12 a total of 69 samples. In Iran Jamshidiet al.2009, was diagnosed 27 Salmonella isolates out of a total of 1125, which is less than what was found in this study. In slow freezing formation large amount from crystal in extracellular, while rapid freezing lead to small intracellular ice crystals formation, Ice crystals large in size and lead to cell damage by deactivate internal structures, cell walls and membranes (Jay, 2005). Obafemi and Davi,1986 found that Salmonellatyphimurum affected by double freeze-thawing with viability reduced by 99.0% and 95.6% respectively.

Gross Lesion:

Haideret al.,2012 after experimentally infected orally with 2 x10^7 CFU dose of S. Pullorum, the gross findings haemorrhagic and congested liver and observed necrotic foci, also found lung congested, edematous and brown coloured, in the ceca lumen found caseous materials semi-solid, cheesy material and button like ulcer and swelling, congestion in spleen. In this study, most birds with virulent S. pullorum challenge did not show prominent gross lesion of pullorum disease. These results are in agreement with Rahul et al. 2015 Other changes in one case the liver included enlarged a bronze discoloration, this similar to result of Naziret et al. 2014

Histopathological changes:

In this study observed in the cecum hyperplasia goblet cells also lymphocytic infiltration and degeneration and desquamation of the epithelium, these observations are similar to those from Naziret et al., 2014. Degenerated villi of intestine Similar microscopic lesions were reported by Haideret al.,2012 Lourenço et al.,2016.

Degenerative necrosis and infiltration of mononuclear cells in liver in present results corresponding with that of Hossainet al.2006 cells are lost its nuclei, inflammatory cells with kupffer cells, extensive necrosis of the hepatocytes similar lesions also reported by Garcia et al. 2010

Passive haemagglutination: PHA test was conducted for determination of antibody titre of the sera of vaccinated and unvaccinated chickens. Control chicks PHA titre of sera samples were recorded as table 3, which was closely related to the findings of Ferdouset al. 2008. In this study the titre of antibodies vaccinated chicks observed the lowest antibody titre was8 and the highest antibody titre was 128, this result is similar to that found by Bhattacharya et al., 2004 and others, where they noted that the titre of the antibodies caused by the vaccination reached the peak.

DTH Results: The results in this study are in agreement with reports in which induction of the DTH reaction in response to Salmonella immunization was shown to be responsible for protective immunity in Salmonella infections Gupta et al. 1996 et al. 2005.
Reference


[4]. Ferdous J (2008). Immunogenicity study of DLS prepared *Salmonella gallinarum* vaccine in comparison to commercially available one in layer chicken. M.S. Thesis submitted to the Department of Microbiology and Hygiene, Faculty of Veterinary Sciences, Bangladesh Agricultural University, Mymensingh, pp. 38–39.


