

Study the clinical and immunological parameters of Rabbits infected by Salmonella Typhimurium

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Abstract

This study included the collection of (100) stool samples from patients ranging in age from 4 months to 40 years suffering from acute and bloody diarrhea, from different hospitals in Iraq for the period 10/2/2021 to 4/5/2022, and these samples were cultured on many media. Facultative, differential and enrichment cultures for the purpose of isolating salmonella bacteria, where (15) isolates of salmonella were obtained after it was diagnosed with the VITEK-2 device and its return was proven (5) isolates of non-typhoidal salmonella (Typhimurium). (40) local rabbits of different ages were used and divided into two groups, where the first group (20) rabbits to estimate the infected dose, the second group (10) rabbits were divided equally and used in experimental infection, as of the control group, and the infected group, where the infected dose was estimated to be equal to (5x10⁷) and injected intraperitoneally For rabbits, where the experimental animals after the injection of the infected dose showed many clinical signs, including lethargy, diarrhea loss of appetite for food and weight loss, in addition to that,. As for the cellular and humoral immunological parameters, when conducting the DTH test, an increase in the thickness of the skin was observed 48 hours after the injection, while the phagocytosis index showed a significant increase in ingestion of yeast, while the IgG test showed an increase in the measurement of antibodies, which reached a height in the second, third and fourth week of experiments.

Key word Salmonella Typhimurium, Rabbits, immunological

1. Introduction

Salmonella is a main health problem worldwide that causes non-typhoidal salmonellosis (NTS) and typhoidal salmonellosis. These two categories of salmonellosis infect millions of cases yearly with important economic losses and even life threatening. (Barrow and Methner, 2013; Manoj *et al.*, 2015). Moreover, the existence of *S. Typhimurium* is risk to public health and persons who have direct contact to sheep and goat (Esmaeili and Rahmani, 2016). In human disease, the clinical pattern of salmonellosis can be divided into four disease patterns namely enteric fever, gastroenteritis, bacteremia and other complications of non-typhoidal salmonellosis as well as chronic carrier state. (Pui *et al.*, 2011). *Salmonella Typhimurium* commonly causes gastroenteritis in immunocompetent persons. However, there is growing evidence to suggest that in certain susceptible populations, such as babies in sub-Saharan Africa and HIV-Positive adults, *Salmonella Typhimurium* infection exhibits as septicemia without any gastroenteritis (Gordon, 2008; Gordon, 2011; Feasey *et al.*, 2012). This report aims to know the clinical symptoms caused by bacteria to experimental animals and their effect on liver and kidney functions, in addition to knowing the cellular and humoral immunological parameters.

2. Material and methods

Samples collection

(100) samples were collected from the stool of pediatric and adult patients aged between 5 months

to 50 years suffering from acute watery and bloody diarrhea, from several hospitals in Iraq, including Balad General Hospital in Salah al-Din, Baquba General Hospital in Diyala and Karma Teaching Hospital in Anbar, for the period between 2/10/2021 to 4/5/2022, where these samples were taken directly to the laboratory and planted immediately after obtaining them from the patients, and (1) gram of feces were added to tubes containing 10 ml of Selenite Broth medium and mixed well, then These tubes were incubated at a temperature of (37°C) and for a period of (24 hours), after that a sample of the resulting microbial growth was taken and planted on selective culture media- microscopy *Salmonella* bacteria appear under the light microscope as Gram-negative bacteria in addition to *Bacillus* and single cells after (18-24) hours after incubation at 37°C. - VITEK examination Using the VITEK device, the final diagnosis of bacterial isolates was made for the purpose of ascertaining their belonging to the non-typhoidal salmonella genus. This device provides 64 biochemical tests, as the diagnosis was made in the Tigris laboratory, and the relevance of 5 isolates of non-typhoidal salmonella bacteria (*S. typhimurium*) was proven.

Animals

A total number of sixty domestic rabbits of both sexes with an age range (10–12) months old and weighing between (1500 and 2000)gm, were obtained from local markets, during the experimental period, They were housed in clean metal cages at room temperature about (22 ± 3, at an experimental animal house in College of

Education/ Al-iraqia University, the rabbits were fed commercial pellet, green food (alpha alpha) and water was supplied. and Animals were adapted for 10 days before starting the experiment, then used in two experiments:

First experiment (I): twenty rabbits were used in this experiment to estimate the infective dose (ID) of the *S. typhimurium*.

Second experiment (II): forty rabbits were used in this experiment to infect the rabbit with the estimated infective dose of *S. typhimurium*

Preparation of *S. typhimurium* infective dose.

Five groups of rabbits (each group include 4 rabbits) these Rabbits had negative fecal bacteriological culture for *Salmonella*, four groups of Rabbits injected intraperitoneal (IP) with one of the calculated diluents (C.F.U./ml) above dilute with 1ml while the last group served as control and injected with 1ml PBS (PH=7.2).

All groups of Rabbits were observed for 3 days to calculate the live and dead rabbits, the infective doses (ID) were estimated by choosing the group of rabbits, which showed clinical signs with no mortality after appearance of clinical signs two rabbits were killed for necropsy finding and isolate *Salmonella typhimurium* from internal organs.

3. Delayed type hypersensitivity test DTH (skin test)

Immunological testes

Cellular immunity

According to Hudson and Hay, (1980), testing the DTH was preformed by skin test as follow, soluble antigen of *Salmonella enteritidis* have protein about 250µg/ml was used to be inoculate intradermally of infected rabbit skin of right side of abdomen and in amount of 0.1 ml. The skin of left side of abdomen was used as control and injected with sterile PBS (pH 7.2) of same inoculum size. The thickness of the skin at the site of inoculation was measured before and after the use of Vernier and calliper, for two rabbits 24, 48 and 72 hrs post injection.

Phagocytic index

The phagocytic index in the blood of infected and control animals was detected according to method described by Weber et al., (1982). The engulfing activity of phagocytes was measured when 250 µl of Hanks solution was mixed with same volume of rabbit blood with heparin. This mixture by 50 µl of killed yeast, then the final suspension was incubated at 37°C for 30 minutes. Using of clean microscopic glass slide, thick smear from the mixture was made, fixed in methanol and stained for 10 minutes with 10% Gimesa. The extra stain was removed by washing with tap water, air dried and subjected to microscopic oil immersion lens testing. The phagocytic index can be calculated using the following equation, when 200 cells were at least be calculated.

in Serum against *Salmonella typhimurium*.

Humoral Immunity Test

Immunoglobulin IgG test in rabbit blood serum

The concentration of IgG in rabbit blood was calculated by using the Enzyme-linked Immunosorbent Assay ELISA technique. Blood samples were collected from both groups A and B at the second, third, fourth, and sixth week after experimental infection. The blood sample was Separate to obtain the serum and freeze it at a temperature of 20°C until the examination is performed.

Ethics Approval

This study was approved by the ethical and research committee of College of Education – AL- Iraqia University.

4. Result

1-The first experiment (estimating the infected dose)

After isolation and diagnosis of bacteria, the infective dose was estimated for experimental animals (rabbits) injected with (1) ml of non-typhoidal salmonella bacteria (*Typhimurium*) inside the peritoneum, which is equal to (5×10^7) as in table (1) , The infective dose used in this study which is in agreement with a study by Reis et al. (2011) and Hung et al. (2009) who reported that ID was 5×10^7 (CFU/ml), where the researchers Blaser and Newman (1982) mentioned that the rate of the infected dose ranges between (10^5 - 10^7) . The peritoneal cavity was targeted for injection in the current investigation. Al-Qaisi (2004) said that the intraperitoneal infection was preferable to the oral dose since the digestive system has several protective factors, such as stomach acidity and the existence of normal intestinal flora. Unlike the peritoneal cavity, which is devoid of such obstacles

Table (1) shows the estimation of the infective dose (ID) of non-typhoidal salmonella bacteria for experimental animals (rabbits)

(Group)	Dose C. F. U./ml	Live	Death
1	1010×5	0	4
2	109×5	2	2
3	108×5	1	3
4	107×5	4	0
5	P B S	4	0

2-The second experiment (Injecting rabbits with the right dose of non-typhoidal salmonella bacteria

When the experimental animals were injected with infected dose, all Rabbits were monitored daily in order to observe clinical signs, as the rabbits of the affected group showed many symptoms, including lethargy, high temperature (fever), loss of appetite, lack of response to stimuli, where the animal's body temperature was higher than 39.5 pm on the first and second day after the injury, then it began to gradually decrease, and one of the signs that was also observed was diarrhea accompanied by mucus, and a change in the consistency of the stool was observed starting from the second day after the infection, and the color of the stool changed from dark brown to black to light greenish brown and then

to watery diarrhea on the third and fourth day, and the stool was containing blood clots on the third day, and frequent urine Hazy color and milky color, these symptoms lasted for three days and the recovery period was completed (6-8) days in addition to shortness of breath and increased consumption of quantities of water that usually these are associated with symptoms of fever and diarrhea. These results were in agreement with the study mentioned by Harab A, (2010) in which the rabbits were injected experimentally with *salmonella* bacteria Al-Kaabi (2000) and Al-Tai (2002) indicated this view in their studies in Baghdad, that most of the salmonella isolates isolated from human diarrhea were serotype (*S. Typhimurium*) The clinical signs of this study were in agreement with the results reported by Ann (2005) that were performed on rabbits injected with *E. coli* bacteria, while the control group did not show any change in behavior or other signs when compared to the infected dose. The dominant cells in the immune response resulting from NTS are neutrophils, These cells cause ruptures in the lining of the intestine, especially in the ileum and colon, by the action of the circulatory cells that produce them, Therefore, animal feces contain pus in addition to blood, The severity of the appearance of clinical symptoms depends on two factors, the first of which is related to the virulence of the salmonella serotype, while the other depends on the factors of the host, and these factors contribute to causing the disease as mentioned (Hohmann, 2001).

Immunological tests

Delayed type hypersensitivity (DTH) test

After conducting a test (DTH) before the injection process (zero time) for both groups (control and infection), the results were (2.23 ± 0.004) for infection group while the control group was (2.23 ± 0.006), where the infected group showed a significant increase at ($P \leq 0.05$) in Skin thickness (the left side) of the experimental animals when compared to the control group.

The increase in skin thickness reached its highest percentage on the second day of infection (after 48 hrs) of the injection, and then the thickness of the skin began to decrease, (72 hours) after the injection, while the control group did not show any change in the average thickness of the skin as shown in the figure (1-6).

Cellular immunity against specific fungal, bacterial, or viral antigens can be assessed by DHT when each of these antigens has been inoculated intra-dermally (Sadaoka *et al.*, 2008). Turk (1980) reported that some inflammatory responses can be seen at the site of inoculation such as thickening of the skin, redness of the skin, and vesicle formation which may be followed by ulceration. The DTH test is used as a diagnostic method for detecting the cellular immune response. Thickening of the skin is caused by the accumulation of too many monocytes and phages at the site where the antigen is present. IFN γ and IL were released from the previously stimulated Th1.

These (chemokines) are chemical agents of inflammatory cells and the esophagus to the site of infection (Mastroeni *et al.*, 1998).

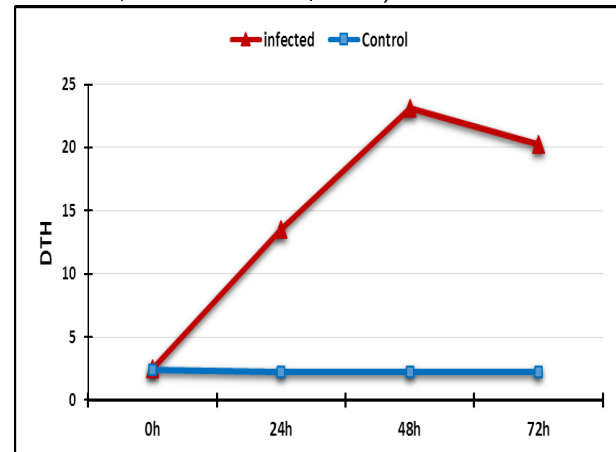


Figure (1-6) Means of skin thickness (millimeters) of infected and control groups in DTH test.

Phagocytosis index test.

The results showed a significant increase in phagocytosis indices in the infected group, which amounted to (36.60 ± 1.7) and there were significant differences at ($P \leq 0.05$) compared to the control group in which the phagocytosis index reached (13.5 ± 1.18), this test where conducting in order to evaluate the work of cellular immunity, especially macrophages, which work on devouring foreign bodies (antigens) entering the body.

Use of killed yeast in phagocytosis assay to stimulate the antimicrobial activity is known to be significant activity against foreign antigens, phagocytosis can be stimulated by (*S. Typhimurium*) thus, this can result in the destruction of bacteria due to the work of phagocytosis, which released some enzymes such as Proteases (Magnusson, 2009)

S. Typhimurium have Surface lipopolysaccharides against pathogens, which have the ability to impair the phagocytosis (Nordhaug *et al.*, 1994), as in the figure (1-7).

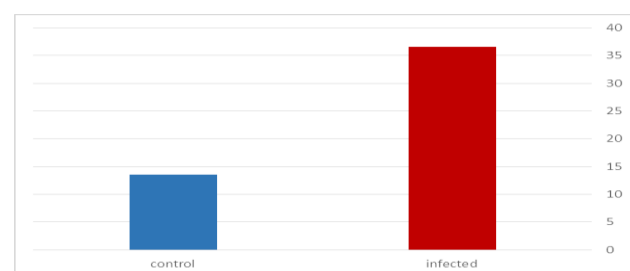


Figure (1-7) phagocytic indices in infected and control groups 3- IgG test

Before infection, all experimental animals (infected and control group) revealed low level of antibodies titers. post immunization, the infected group revealed an increase in serum antibody titer at the (second, third, and fourth) weeks post-immunization. In this group, a decline in antibody titer was recorded towards the termination of the study. there were significant differences at ($P \leq 0.05$) compared to the control group, while the control group did not show significant differences throughout the experiment

period.

The results of our study agree with a study conducted by Shallal (2011) in which mice infected with *Salmonella Mbandaka*. But Davidson (1987) reported a higher efficacy of infected group in triggering production of specific antibody and enhanced neutrophil ability to destroy *S. Typhimurium*. As in figure (1-8).

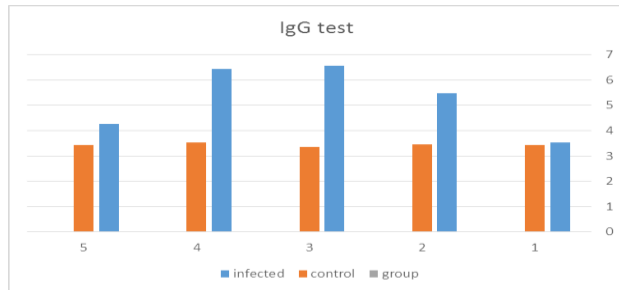


Figure (1-8) Serum antibody titers in infeced and control groups of ewes as determined by ELISA test at different times.

5. Conclusions

The infected dose of this bacteria was (5×10^7 C.F.U) and that this bacteria has the ability to cause many systemic symptoms, including severe diarrhea and loss of appetite for food. Disease events in rabbits are associated with immune stimulation

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