

Experimental Salmonellosis in Dogs: Clinical, Bacteriological and Pathological studies

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Abstract: This study was conducted to investigate the clinical and pathological effect of Salmonella Kiel that isolated from apparently normal dog on experimentally infected dogs. Ten mongrel dogs of 6 months old were used in this study. The dogs divided into two groups. The first group kept as a control negative group, while the second group was orally given 1×10^9 CFU/ml of Salmonella Kiel (infected group). Animals euthanized after 30 days. One dog of the infected group showed a rise in rectal temperature (40°C) within 24 h, followed by depression, loss of body weight and diarrhea and then died after 8 days. Salmonella Kiel was isolated from rectal swabs, intestine, liver, kidneys and gall bladder of experimentally infected animals during and at the end of the experiment and identified by PCR. Microscopically, small and large intestine of experimentally infected animals revealed necrotizing enteritis. Histopathologic examination of liver of infected animals showed vacuolar degeneration of hepatocytes with presence of small foci of coagulative necrosis infiltrated with mononuclear cells (paratyphoid nodules). Kidneys of inoculated dogs revealed coagulative necrosis of lining epithelium of renal tubule in both cortex and medulla. In conclusion, apparently normal dogs can harbor Salmonella and release it in their feces, so they act as a source of infection to animals and human.

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1. Introduction:

Infectious enteric pathogens have long been recognized as a significant problem owing to their pathogenicity potential to animals and their zoonotic risk to humans (Tsai et al., 2007).

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 diarrhoea and death in numerous animal species and human beings (Ojo and Adetosoye, 2009).

Salmonellosis as a zoonotic disease has become a worldwide problem, it is believed that the incidence of this disease is increasing in both man and animals specially dogs. In most dogs the infection occurs in latent form. Infected dogs tend to shed salmonella in their faeces and saliva for prolonged time (Verma et al., 2007).

Infection in dogs is most often caused by consumption of infected food, contact with infected faeces or other environmental components, and scavenging (Apanavicius et al., 2007).

The incidence of Salmonella infection including a carrier state in dogs is very important to public health, because dogs are usually reared in contact with humans (Sato and Kuwamoto, 1999).

Aim of the work: This study was planned to investigate the effect of isolated Salmonella kiel from apparently normal dog (latent infection) on experimentally infected animals of the same species to detect the carrier state of salmonellosis.

2. Materials and methods:**I- Bacterial strain:**

Strain was previously isolated from apparently healthy, 1.5 years old male German shepherd dog which was imported recently from Netherland. Rectal swab from this dog was enriched on selective media (selenite f broth and tetrathionate broth) for 18 h at 37°C . Sample were then plated onto MacConkey's agar and S-S agar plates and incubated for 24 h at 37°C . The transparent non-lactose fermenting medium sized colonies were subjected to identification by the following biochemical tests: triple sugar iron agar (T.S.I.), citrate agar and urea agar slants. Identification also confirmed by PCR assay.

Serotyping: Once salmonellae were identified, serotyping was performed at Ministry of Health Laboratories according to the Kauffmann-White scheme using a commercial antiserum kit (Difco).

II-Animals:

Ten mongrel female dogs, about six months old were used in this study. Dogs were acclimatized for

seven days before the onset of the study, they were fed dry food. The drinking water was offered ad libitum.

III-Experimental design:

Ten dogs were examined bacteriologically for salmonella by rectal swabs and proved free of salmonella were used in this experiment.

Dogs were randomly divided into two groups. The first group (contain five dogs) served as a control. The second group (contain five dogs) was given a single dose of Salmonella kiel inoculum. The inoculum was administered orally at a dose of 10 ml of 1×10^9 CFU/ml.

The rectal temperature and clinical signs were recorded. All dogs of the first and the second groups were euthenized at the end of the experiment (after 30 days).

IV- Isolation and identification of recovered Salmonella isolate:

Rectal swabs were collected daily from dogs till the end of the experiment. After 30 days samples from spleen, liver, kidneys, intestine and gall bladder were collected for bacteriological examination. Each sample was treated as mentioned before for isolation, identification and serotyping of Salmonella species.

V- PCR assay:

The presence of the *invA* gene was verified by PCR analysis.

DNA extraction: DNA samples for PCR analyses were obtained from suspicious cultures. One colony was selected, seeded on BHI broth (5 mL) and incubated at 37°C for 12 h. The culture was centrifuged and the sediment resuspended in 200 μ L phosphate buffered saline 0.05M, pH 7.2 (PBS), then adding of 10mg/ml lysozyme and incubated for 1 h at 30°C, followed by addition of 200 μ g/mL proteinase K and incubation at 55°C for 1 h. Then, 300 μ L 10% sodium dodecyl sulphate (SDS) were added and incubation was performed at 65°C for 10 minutes; 600 μ L chloroform and 400 μ L protein precipitation solution (potassium acetate 5M and acetic acid 11%) were also added. The tube was then centrifuged at 10,000 rpm for 10 min and the supernatant was carefully transferred to a clean tube then adding of 1 mL absolute ethanol for later centrifugation for 5 min. The sediment was added to 1 mL ethanol 70% and centrifuged for 2 min. After the sediment was dried, DNA was eluted in 50 μ L Tris-EDTA and incubated at 65°C for 5 min. The extracted DNA was quantified in a Nanodrop spectrophotometer (Thermo).

PCR: For PCR, 10 μ L DNA template were mixed with 2.5 U Taq DNA polymerase, 50 pmol of each primer, 200 μ M deoxynucleoside triphosphate, 1.5 mM MgCl and PCR buffer 1X in a final volume of 25 μ L. Following an initial denaturation at 94°C for three minutes, the material was subjected to 35 thermal cycles of 94°C (denaturation) for one minute, 56°C

(annealing) for one min, and 72°C for 40 seconds. The reactions were carried out in a thermocycler. A 5 μ L volume from each reaction was subjected to electrophoresis on 0.8% agarose gel, stained with ethidium bromide/SybrGold and later visualized in a transilluminator (Ultra Violet Products). The amplification of an 284 bp fragment, corresponding to *invA* gene, was expected. For the amplification of *invA* gene, the following primers were used:

invA R: 5' GTGAAATTATCGCCACGTTCCGGGCAA 3'

invA F: 5' TCATCGCACCGTCAAAGGAACC 3'

VI-Postmortem and histopathological examination:

At the time of euthenization, dogs were subjected to postmortem examination to detect any abnormal gross changes. Tissue specimens from intestine, liver and kidneys of dogs were collected, fixed in 10 % neutral buffered formalin, processed and embedded in Paraffin wax, sectioned at 4 μ m and stained with Hematoxylin and Eosin (**Bancroft and Gamble, 2008**) and examined under an Olympus microscope (Olympus, Japan). Also samples were collected from dead animal handled as before.

3. Results

Concerning group 1 (Control group), no abnormal clinical signs, pathological changes or Salmonella microorganism were detected.

In group 2 (infected group) the results were recorded as follow:

Clinical signs:

One dog of the infected animals showed a rise of rectal temperature (40°C) within 24 h, followed by depression, loss of body weight and diarrhoea, this dog died after eight days of the experiment. Other animals in the same group did not show any apparent clinical signs.

Bacteriological findings:

Shedding of Salmonella kiel in faeces began after 48 hours, then shedding became sporadically and continued till the end of the experiment. The organism was isolated from liver, kidneys, intestine and gall bladder of the dog after death at day 8th (case 2), and other experimentally infected animals at the end of the experiment (day 30th) as shown in table (1).

PCR findings: Salmonella strain was tested by PCR and showed amplification with a molecular length of 284 bp (Fig. a), corresponding to the *invA* gene.

Postmortem and histopathological findings (after 30 days):

No observable gross abnormalities were detected in examined organs of infected dogs.

Small intestine of infected animals developed necrotizing enteritis by microscopic examination, in which there was coagulative necrosis of the enterocytes lining intestinal villi with complete fusion of these villi at areas of necrosis. Lining epithelium of intestinal glands also showed coagulative necrosis with atrophy

or complete disappearance of some intestinal glands. Lamina propria and submucosa of small intestine infiltrated with mononuclear inflammatory cells (lymphocytes, macrophages and plasma cells) with presence of inflammatory edema dispersed submucosal connective tissue (Fig. b1 & b2).

Microscopic examination of colon of experimentally infected animals showed focal area of necrosis in submucosa with infiltration of lamina propria and submucosa with mononuclear inflammatory cells (lymphocytes, macrophages and plasma cells) (Fig. b3).

Histopathological examination of liver of infected animals showed vacuolar degeneration of hepatocytes with presence of small foci of coagulative necrosis infiltrated with mononuclear cells (paratyphoid nodules). There is activation of Kupffer cells in all examined cases (Fig. b4 & b5). Portal area showed fibroplasia with congestion of portal blood vessels (Fig. b6).

Kidneys of inoculated dogs revealed coagulative necrosis of lining epithelium of renal tubule in both cortex and medulla with congestion of interstitial blood vessels (Fig. b7 & b8).

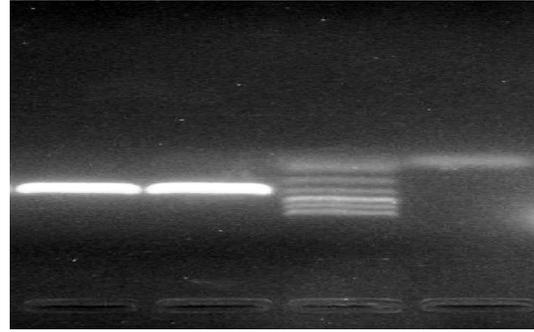


Fig. a: PCR finding

Agarose gel stained with ethidium bromide with polymerase chain reaction (PCR) products of Salmonella isolate, showing amplification with a molecular length of 284 bp.

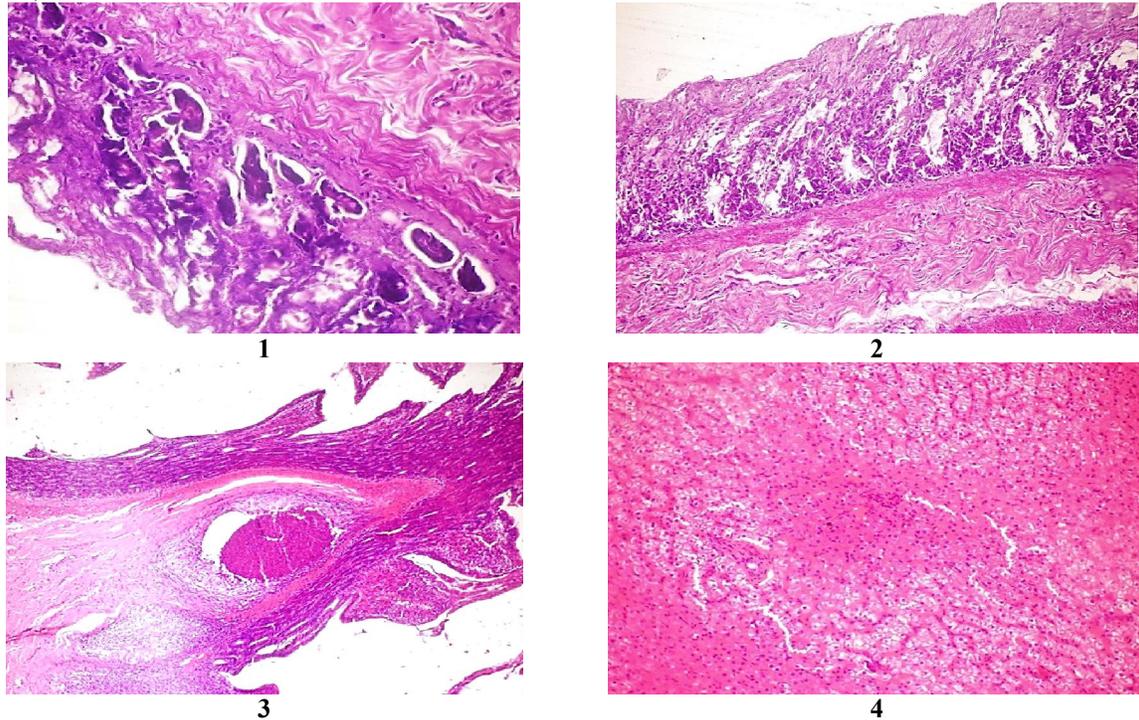


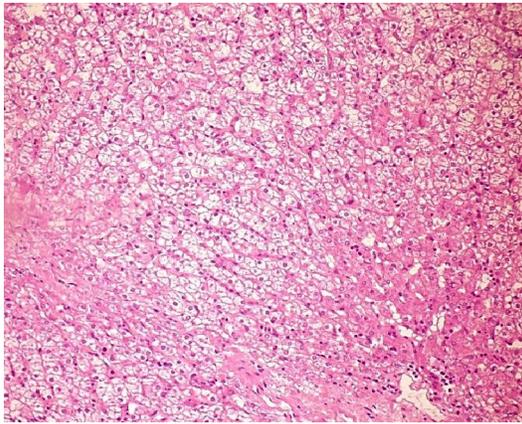
Fig. b: Histopathological finding

1: Micrograph of small intestine, dog infected with Salmonella kiel. Note coagulative necrosis of the enterocytes with complete fusion of intestinal villi. Lining epithelium of intestinal glands also showing coagulative necrosis with atrophy of some intestinal glands, there is also inflammatory edema dispersed submucosal connective tissue. (H&E X 200)

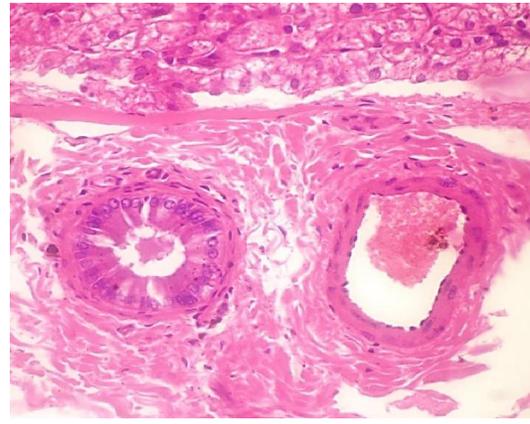
2: Micrograph of small intestine, dog infected with Salmonella kiel. Showing necrosis of the enterocytes. Lamina propria and submucosa infiltrated with mononuclear cells. (H&E X 200)

3: Micrograph of colon, dog infected with Salmonella kiel. Showing focal area of necrosis in submucosa with infiltration of lamina propria and submucosa with mononuclear inflammatory cells. (H&E X 200)

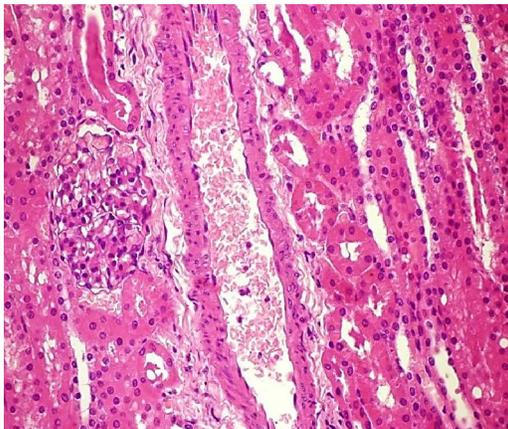
4: Micrograph of liver, dog infected with Salmonella kiel. Notice vacuolation of hepatocytes and small foci of coagulative necrosis that infiltrated with mononuclear cells (paratyphoid nodules). (H&E X 200)



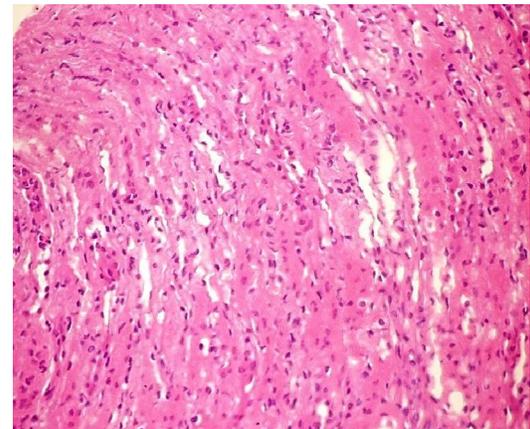
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5: Micrograph of liver, dog infected with *Salmonella kiel*, showing vacuolar degeneration of hepatocytes with activation of Kupffer cells. (H&E X 200)

6: Micrograph of liver, dog infected with *Salmonella kiel*, showing fibrosis in portal area with congestion of portal blood vessels. (H&E X 400)

7: Micrograph of renal cortex, dog infected with *Salmonella kiel*, showing coagulative necrosis of lining epithelium of some renal tubule with congestion of interstitial blood vessel. (H&E X 400)

8: Micrograph of renal medulla, dog infected with *Salmonella kiel*. Note coagulative necrosis of lining epithelium of medullary renal tubule. (H&E X 400)

Table (1): Recovery of salmonella from organs of infected dogs.

	spleen	Liver	Kidneys	intestine	gall bladder
case 1	—	+	—	+	+
case 2	—	+	—	+	+
case 3	—	+	+	+	+
case 4	—	+	—	+	+
case 5	—	+	+	+	+

4. Discussion:

Multiple serotypes of salmonellae commonly exist in dog populations. The prevalent serotypes of salmonellae in dog populations are quite variable among different countries and at different times within the same country (Tsai et al., 2007).

Salmonella species can be a cause of enteritis and diarrhea in dogs, but can also be isolated from the

feces of clinically normal dogs. This can confuse the interpretation of the significance of *Salmonella* isolation, particularly in diarrheic animals (Cantor et al., 1997).

This experiment revealed that non-diarrhoeic dogs can harbor *Salmonella*, so they act as reservoirs with shedding of salmonellae in their feces. These

pathogens in their feces may ultimately infect other animals by contaminating the environment.

In this study one dog from the infected group showed a rise in temperature (40°C) within 24 h, followed by depression and loss of body weight and this result were similar with that recorded by **Clarvet, 1985**. Diarrhoea began to appear during the beginning of the second week and this result was observed by **Radostitis et al., 2000**

The dog died after 8 days from the beginning of the experiment.

Other animals in the same group did not show any apparent clinical signs, but only shedded salmonella in their feces till the end of the experiment.

Most dogs are asymptomatic when they act as reservoirs shedding salmonellae in their feces (**Tsai et al., 2007**).

Salmonella kiel was isolated from rectal swabs, liver, kidneys, intestine and gall bladder of experimentally infected animals during and at the end of the experiment (day 30th) and this result was compatible with that reported by **Frizzo et al., 2012**.

Salmonella strain was tested by PCR and showed amplification with a molecular length of 284 bp, corresponding to the *invA* gene and this result was compatible with that recorded by **Li et al., 2012**. Molecular tests have been designed for the detection of many virulence genes and are often the most sensitive methods for detecting them (**KILIĆ et al. 2007**).

There were no observable gross abnormalities in examined organs of infected dogs.

The intestine of the infected animals developed necrotizing enteritis by microscopic examination, in which there was coagulative necrosis of the enterocytes with complete fusion of intestinal villi. Lining epithelium of intestinal glands also showed coagulative necrosis with atrophy of some intestinal glands. Lamina propria and sub mucosa infiltrated with mononuclear cells and this result was agreed with **Orr et al., 1977**.

The oral entry of Salmonella, proliferation of this microorganism in the small intestine, and its rapid penetration into the lamina propria cause edema, macrophage and lymphocyte proliferation. This is accompanied by expansion of the central lacteals, and generates a sharp decline in apical enterocytes while provoking a proliferative reaction in the bottom of the crypts (enteritis regenerative). This process is rapidly decompensate, causing atrophy and fusion of the villi. The bacteria also invade regional lymph nodes, leading to macrophage recruitment. The same happens in submucosa veins, producing phlebitis and thromboembolism. Circulatory disorders lead to irreversible damage of the villi with apical necrosis (necrotic enteritis) (**Frizzo et al., 2012**).

Salmonella infection resulted in increased macrophage infiltration in ileal tissue. Macrophage infiltration was expected following infection because of their role in phagocytosis and pathogen killing. Enterocyte sloughing indicates epithelial damage (**Apanavicius et al., 2007**).

Histopathologic examination of liver of infected animals showed vacuolar degeneration of hepatocytes with presence of small foci of coagulative necrosis infiltrated with mononuclear cells (paratyphoid nodules), and this result was similar to that reported by **Frizzo et al., 2012**. There is activation of Kupffer cells in all examined cases. Portal area showed fibroplasia with congestion of portal blood vessels. Kidneys of inoculated dogs revealed coagulative necrosis of lining epithelium of renal tubule in both cortex and medulla with congestion of interstitial blood vessels.

Oral administration of Salmonella species causes proliferation of microorganism in the small intestine. Then it invades regional lymph nodes and spread through blood circulation and the lymphatic system causes septicemia with involvement of liver and kidneys **Orr et al., 1977**

It is concluded that, apparently normal dogs can harbor Salmonella, release it in their faeces and considered as a latent source of infection to other animals and human.

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