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Salmonella mbandaka isolated from human: Clinical and gross pathological studies in experimentally infected mice

Zinah Shakir Shallal [1*], Afaf Abdulrahman Yousif [2] and Mahdi Saber Al-Deresawi [1]

[1] Department of Biology / College of Science/Wasit University
[2] College of Veterinary Medicine. / Baghdad University

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***Corresponding author:**
Email address:
zish33@yahoo.com

Abstract

This study was intended to study the clinical, bacteriological and gross pathological aspects of *Salmonella mbandaka* isolated from human in experimentally infected mice. The infective dose (ID) of *S. mbandaka* was estimated in 48 mice, about 6-8 weeks old of both sexes. Clinical and gross pathological features of *Salmonella mbandaka* were studied in 28 mice. These were divided randomly into 2 groups: Group A" included 14 mice and were inoculated orally with *S. mbandaka* ID that was determined earlier , and Group B with 14 mice and were inoculated orally with phosphate buffer saline and considered as a control group. The infective dose (ID) of *S. mbandaka* in mice was (1.3×10^7 cells), that showed the clinical signs of Salmonellosis without mortality. All mice in the control group (Group B) were appeared healthy during the experimental period. However, the results of experimental study revealed that the most important clinical signs were observed in the orally infected mice with *S. mbandaka* (Group A). These signs were included mild diarrhoea, dullness (24 hours post inoculation), fever and anorexia. In addition, the thirst and decrease in the activity were prominent at 48 hours post infection. However, complications such as epididymitis and meningitis were observed at 5th and 6th days post infection respectively in two mice. The gross pathological changes were observed on the small and large intestine at 24 hours and 48 hours post infection and characterized by variable degrees of hyperaemia and gelatinous appearance due to accumulations of mucus. At 72, 96 and 144 hours post infection, the liver and spleen were severely enlarged and congested together with petechial haemorrhages mainly on the spleen surface. In addition, the gall bladder was also engorged at 48 hours and the kidneys were swollen at 72 and 144 hours. Moreover, the heart revealed the flabby appearance at 120 hours and 2 weeks post infection. In conclusion, this study revealed that *Salmonella mbandaka* was able to cause systemic infection with complications such as meningitis and epididymitis. In addition to prominent gross pathological changes. The authors recommend more studies on *Salmonella mbandaka* to investigate another pathogenic properties.

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Introduction

Salmonella is the causative agent of a wide variety of diseases and disease syndrome in human beings, different animals and birds. It remains as a serious problem with public health significance throughout the world (Begum *el al.,* 2008). *Salmonella* can colonize and cause diseases in a variety of food-producing and non-food-producing animals. Within this genus, more than 2,500 serovars have been described (Graziani *et al.,* 2008). Since, these pathogens are transmitted primarily through contaminated food or water, the presence of strains in food animals and ultimately in raw meat products has important public health implications (Butaye *et al.,* 2006). Christensen *et al.,* (2011) isolated *Salmonella mbandaka* from flocks of geese, and approved its highly diverging colonization dynamics. One flock was intermittently colonized and appeared after 9 weeks without dissemination with multiple serovars appearing at later stages. In human, enterocolitis caused *by Salmonella,* is characterized by sudden onset of diarrhea, which may be bloody, nausea, vomiting, abdominal pain, and headache (Paul and Blake, 2001). The clinical manifestations of typhoid fever is hepatosplenomegaly and progressive fever that is often sustained at temperatures between 38.8 C^0 and 40 C^0 (Parry *et al.,* 2002). Fever or sweats were noted among 95% and splenomegaly among 38% of 100 Malawian adults at initial presentation with NTS bacteraemia (Gordon *et al.,* 2002).

In animals, the enteric form of salmonellosis is the commonest clinical manifestation. However, the wide range of clinical signs are acute septicemia, abortion, arthritis, necrosis of extremities and respiratory disease. Many animals may also be infected but didn't show any clinical illness (OIE, 2010). The domestic, young and pregnant animals are the most susceptible and often presenting as a bloody or profuse watery diarrhoea with pyrexia however, the signs and lesions are not pathognomonic (Wary and Wary, 2000).In Iraq, S*almonella mbandaka* was isolated for the first time from stool samples of diarrheal children by Al- Talib, (2011). Moreover, the histopathological changes in experimentally infected mice *with Salmonella mbandaka* have been reported previously (Shallal, 2011; Shallal *et al.,* 2013). However, clinical signs and gross pathological changes haven't presented. Therefore, this study was designed to study the clinical, bacteriological and gross pathological aspects of *Salmonella mbandaka* isolated from human in experimentally infected mice.

Materials & Methods

Experimental animals

A total of 76 mice (BALB/c) of both gender with age range from 6 to 8 weeks old, were used in this study. The mice were obtained from the (National Centre of Researches and Drugs Monitor in Baghdad) and adapted for two weeks before experiments. Forty eight out of 76 mice were used to determine the infective dose (ID) of the *Salmonella mbandaka.* While, the rested 28 mice were divided randomly into 2 groups each with 14 mice. The first group were inoculated orally with the *Salmonella mbandaka* (group A) and the second group were drenched orally with 1 ml of phosphate buffer saline and considered as control group. Details regarding the experiments are as follow:

Preparation of the bacteria

Salmonella mbandaka was isolated from child, who was suffering from severe diarrhoea, fever and dehydration. Isolated *Salmonella* were identified and confirmed as *Salmonella mbandaka* in the Central Public Health Laboratories (National Centre of *Salmonellae* in Baghdad/ Ministry of health).

Determination of Infective dose (ID)

The ID doses of *Salmonella mbandaka* were prepared according to (Shallal, 2011). Each five colonies of *S. mbandaka* were removed from brain heart infusion agar and inoculated in 10 ml of Brain heart infusion broth at 37 ˚C for 18 hours, then centrifuged in cooling centrifuge (8000) rpm for 15 minutes then the sediment was washed three times with PBS (pH=7.2) and suspended with 1 ml of PBS and diluted at tenfold dilution (10^{-1},10^{-2}.10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8},10^{-9} and 10^{-10}).The viable count of the bacteria in each diluent was made according to method of Miles and Misra, (1938) and dilution which contain (1.3×10^{7} C.F.U/ml) was consider as infective dose .

Experimental infection in mice

Experimental infection was done in 28 mice. These mice were divided randomly into 2 groups:
Group A: This group were included 14 mice inoculated orally with (1.3×10^{7} cells) as ID of the *Salmonella mbandaka*.
Group B: This group were included 14 mice and drenched orally with 1 ml of phosphate buffer saline (PBS, pH=7.2) and considered as control group.
All experimental mice were observed daily and clinical and vital signs were reported for each mouse.

Ethical approval

This study was approved by the ethical and research committee of Veterinary Medicine College/University of Baghdad.

Statistical analysis

Chi square was conducted to determine the statistical differences among the tested groups by using SPSS statistical program (ready–made statistical design).

Result and discussion

Estimating the infective dose of *Salmonella mbandaka*

The results of this study revealed that the infective dose (ID) of *Salmonella mbandaka* in mice was (1.3×10^{7} cells). The ID was estimated by calculating the dead and alive mice in each group during (30) days of the experiment (Table 1). The infective dose of *S. mbandaka* determined in this study was similar to the infective dose of other non-typhoid *salmonella*, that mentioned by other researcher previously (Al-Naqeeb 2009). He found that the ID of *S. hadar* in mice was (1.5×10^{7}). However, this dose was lower when

compared with Al-Talib, (2010), who recorded that the ID of *S .newport* in rabbits was (2×10^8 C.F.U. /ml). The results also compatible with Blaser and Newman, (1982), who referred that the infective dose ranges between 10^5–10^{10} cells, and also with Al-Mansory (2009), who recorded that ID of *Salmonella enteritidis* in rabbits was (2×10^8 C.F.U./ml).

Table (1): The results of estimating of ID of *S. mbandaka* in mice.

Groups	Dose (cells)	Alive	Dead	Total alive	Total dead	Percent mortality
1	1.3×10^{11}	0	8	0	20	**100 %**
2	1.3×10^{10}	2	6	2	12	**81 %**
3	1.3×10^{9}	4	4	6	6	**50 %**
4	1.3×10^{8}	6	2	12	2	**18 %**
5	1.3×10^{7}	8	0	20	0	**0 %**
6	1.3×10^{6}	8	0	28	0	**0 %**
Total No. of mice48 = (No. of mice in each group = 8)						

Clinical signs and gross pathological changes in mice experimentally infected with ID of *S. mbandaka*.

Clinical signs

Twenty four hours post infection, all mice in group (A), which infected orally with (1.3×10^7 cells) showed dullness and tending to aggregate in one place. These signs refereed that the mice suffered from fever, loss of appetite, thirst (when compared with quantities of consumed water of the control group), decreased activity and very mild diarrhoea. The color of faeces was changed from dark black to light yellow and paste in consistency (in comparison before drenching and with faeces of the mice in the control group). These changes were appeared after the 2nd day of infection and continued for 5th day. Complications such as redness, congestion and enlarged in the genital organ were observed in one mice (male) (Figure 1). Nervous sings such as head shaking and half paresis were also appeared in one mice at the 6th day post inoculation. This mouse was dead after 24 hours post appearance of nervous symptoms (Figure 2). The observed clinical sings were disappeared at the 9th day and the mice were returned to their normal condition.

Figure (1): Shows one mouse (male) from group A that revealed redness, swelling and congestion of genital organ at 5th days post infection.


Figure (2): Shows one mouse from group A that revealed nervous sings at 6th days post infection.

All mice in the 2nd group (B) (control group) did not show any clinical signs or disorders during the period of the experiment.

The clinical signs, which were appeared on the mice in group A in this experiment, was similar to that described previously by Monack *et al.*, (2004) , who inoculated the mice orally and approved that this route of infection can cause systemic infection and revealed signs of illness, such as ruffled fur and malasia. These results revealed that *Salmonella mbandaka* can infect the mice and cause Salmonellosis as recorded by others (Bohnhoff *et al.,* 1954; Ruitenberg *et al.,* 1971). The complications such as meningitis and epididymitis, which observed in two infected mice, are in agreement with that mentioned previously by Al-Naqeeb, (2009). Al-Naqeeb, (2009), described the similar clinical signs in experimentally infected mice with *S. hadar* such as dullness, fever, loss of appetite, thirst, diarrhoea, in addition to complications such as meningitis and epididymitis. The clinical signs that observed in this study are also in compatible with study of Harab, (2010) on rabbit infected with *S. hadar* and revealed the same clinical signs.

Gross pathological features

Post mortem changes were investigated in the experimental mice. The mice (2 mice in each time) were euthanized at (24, 48, 72, 96, 120, 144 and 168) hours post infection and were examined for gross pathological changes.

The results of post mortem changes appeared on the infected mice are as follows:

At 24 hours, slight significant gross lesion were appeared on infected mice, however, - at 48, 72, 96, 120,144 and 168 hours post inoculation, all mice showed marked intestinal alterations of catarrhal enterocolitis. In addition, the intestine were flaccid, thin-walled and filled by clear to yellow watery contents with variable amounts of mucus. The lesions were most severe in the mice killed at 24 and 48 hours (Figures 3 and 4).

The other post mortem changes that appeared on the experimentally infected mice, were various degree of hepatomegaly with congestion (Figure 4, 5), engorgement of the gall bladder (Figure 4) and splenomegaly that revealing surface petechial hemorrhage (Figure 6). The enlargement of liver, gall bladder and spleen were continued until 8 weeks

(experimental period) post infection in the survival mice. The heart was flabby mainly at 120 hrs and 2 weeks (Figure 7). The kidneys were also swollen and congested, which might be occurred due to toxemia (Figure 8). All mice in the control group (B) were euthanized with groups (A) and revealed no abnormality in their internal organs at necropsy (Figure 9).

The post mortem findings reported in this study are resemble to previous observations mentioned by Al-Naqeeb, (2009), who noticed that there was a varying degree of intestinal congestion with a presence of mucus in the infected mice with *S. hadar*. In addition, liver and spleen were also swollen severely and congested together with petechial hemorrhages on its surface. Moreover, the gall bladder was enlarged ,the kidneys were swollen and the heart appeared flabby.

The gross lesions reported in this study are in agreement with previous studies Monack *et al.,* (2004), who reported pronounced splenomegaly in mice infected with *S. Typhimurium* , and Peters *et al.,* (2004), who found splenomegaly predicted NTS bacteremia among hospitalized adults in Malawi. Previous study mentioned that both liver and spleen were involved in *salmonella* infection (Lu *et al.,* 2010). The measurement of spleens and livers weights after various period of infection revealed, a significant increase in the spleen weight in all *salmonella* infection groups at week 3 of infection.

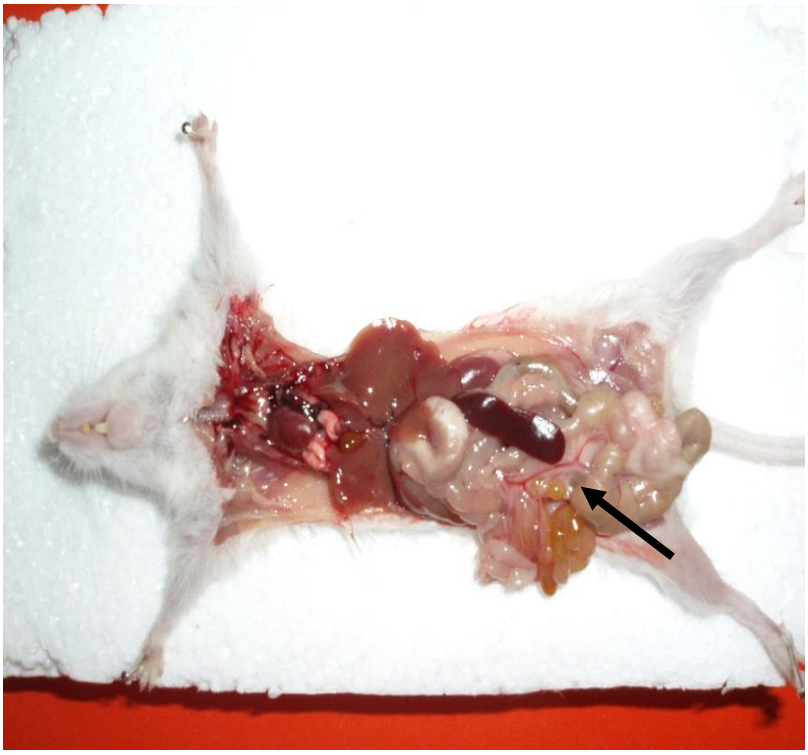

Figure (3): Shows the macrscopical lesions in the abdominal viscera of the infected mice with *S. mbandaka*, including non-significant gross lesion (NSGL) except slight congestion and odema in intestine (Black arrow) at 24 hours post infection.

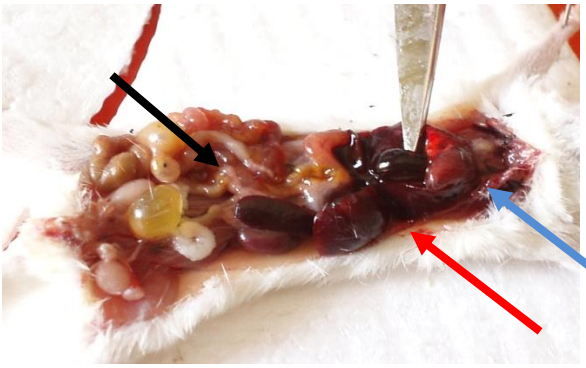

Figure (4): Shows the changes in the abdominal viscera of the infected mice including: congestion, edematous intestine (black arrow) with hyperplasia of mesenteric lymph node and hepatomegaly (red arrow) and enlarged gall bladder (blue arrow) at 48 hours post infection.

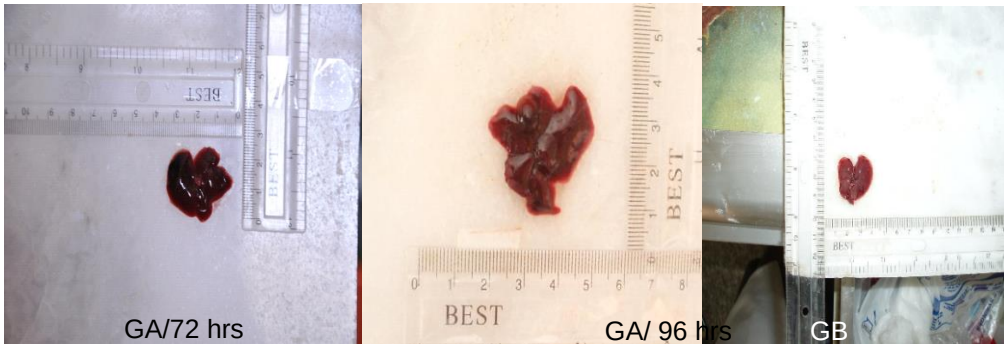

Figure (5): Shows the livers of three mice from both groups after 72, 96 hours post infection. Marked hepatomegaly and congestion of the livers of group (A) mice were observed in compare with control mouse.

Group (A) After 72 hours Post infection. Group (A) After 96 hours Post infection Group (B)

Another study Al-Mansory, (2009) recorded that the gross pathological changes in rabbits infected experimentally with *S. enteritidis*, were characterized by a variable degree of congestion and petechiation of multiple organs and congestion in large intestine. In addition, small intestine were flaccid and filled by yellow watery contents with high amount of mucous, enlargement of payer's patches, engorgement and necrotic foci in liver, enlargement and darkness of gall bladder, enlargement of spleen, congestion and oedema of the lung, focal hemorrhages**,** and enlargement of kidneys .

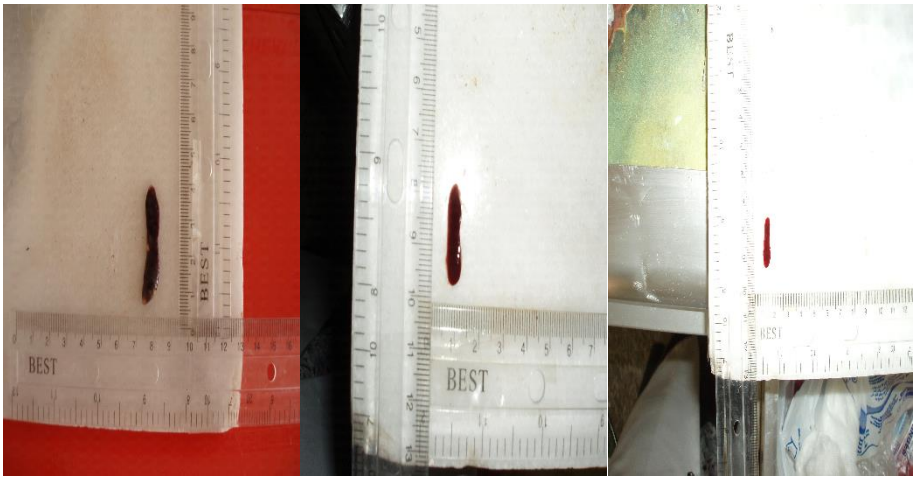

Figure (6): Shows the spleens of three mice from the both groups after 96,144 hours post infection. Marked splenomegaly and congestion in the group (A) mice in compare with control mouse.

Group (A) after 96 hours Post infection. Group (A) after 144 hours Post infection Group (B)

Figure (7): Shows the hearts of three mice from the both groups after 120 hours and 2 weeks post infection. The heart of group (A) mice was flabby on the contrary from control mouse.

Group (A) After 120 hrs Post infection Group (A) After2 weeks Post infection Group (B)

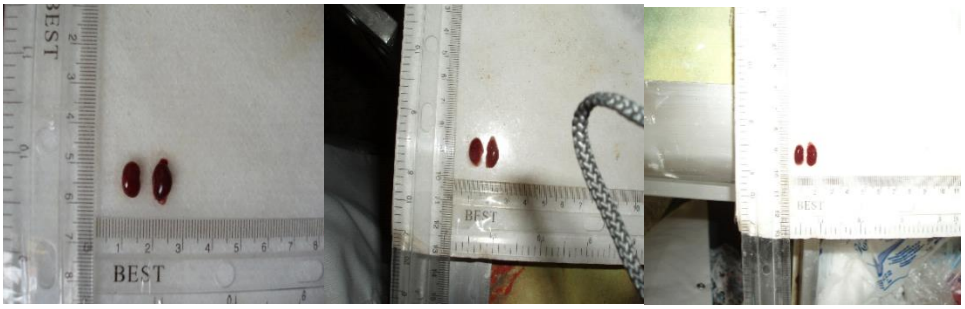

Figure (8): The kidneys of three mice from both groups after 72, 144 hours post infection shows enlargement of the kidneys of group (A) mouse in compare with control mouse.

Group (A) After 144 hrs Post infection Group (A) After 72hrs post infection Group (B)

In conclusion, this study revealed that *Salmonella mbandaka* was able to cause systemic infection in experimental mice. In addition, some complications such as meningitis and epididymitis occurred in some infected mice. *The authors recommend to do more studies on this Salmonella mbandaka* isolates to investigate more pathogenic properties regarding pathogenicity.

Figure (9): Shows the internal organs of mice in group (B).

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