RESEARCH ARTICLE

Outbreak of Salmonellosis in a Laboratory Swiss Albino Mice Colony

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Abstract

A colony of Swiss albino mice was presented with signs of dullness, anorexia with ruffled coat, hunched posture, varying degrees of conjunctivitis and diarrhea. Mice tissues, fecaes, feed, water and bedding material samples were collected for bacteriological examination. Necropsy examination of the mice revealed hepatospleenomegaly, white foci in liver, congestion in kidney and intestines filled with mucoid exudates. The most common histopathological finding was hepatic and splenic necrosis. The bacterial analysis of mice tissues and fecal samples revealed pure culture of *Salmonella* Typhimurium and serotyping confirmed *Salmonella enterica* serotype Typhimurium 4, 12:i:1, 2. The antibiotic sensitivity test revealed that the isolate was highly sensitive to amoxyclav and fluroquinolones and was resistant to aminoglycoside. The mice colony was not treated with antibiotics but was euthanized. From our experience of disease outbreak we inferred that routine screening of animals and gross necropsy findings are very helpful in early diagnosis of disease.

Key words: Mice, Outbreak, *Salmonella* Typhimurium

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Introduction

Salmonella is a Gram-negative, non-spore forming, non-capsulated, intracellular and facultative anaerobic rod-shaped bacterium belonging to family *Enterobacteriaceae*. The genus comprises two species, *S. bongori* and *S. enterica* (Tindall *et al.*, 2005), which include more than 2,600 serovars based on Kauffmann-White scheme (Popoff *et al.*, 2003). All are motile with long, peritrichous flagella, except two serovars, *Salmonella* serovar Pullorum and Gallinarum (Grimont *et al.*, 2000).

Laboratory mice suffer from number of bacterial infections and salmonellosis is one among the most common bacterial infection in mice (Walker *et al.*, 2023). *Salmonella* Typhimurium is the most common serotype of *Salmonella* genus infecting laboratory mice; however there are reports of other serotypes causing epizootics or latent infection in mice. Ingestion of contaminated feed & water and contact with contaminated bedding & animal handler are the major modes of transmission of bacterium (NRC, 1991). Reports of natural outbreaks of disease are rare in the literature (NRC, 1991), probably because most infections are asymptomatic in normal hosts. The severity of infection is dependent largely upon the dose and route of infection, bacterial serotype, the strain of host mouse as well environmental factors. The infection is characterized initially by diarrhea or soft stools, sudden deaths, anorexia, and cachexia.

The initial outbreak of salmonellosis soon subsides into a chronic form of the disease wherein the organisms are shed in fecal material. The adult carriers often appear healthy, but suckling and growing mice show great variation in body weight and rates of gain. Broad spectrum antibiotics can cure the illness and reduce the mortality in epizootics of Salmonellosis in mice colony but have failed to cure chronically infected animals (Grassl *et al.*, 2008). The most common method to eliminate the infection is by euthanizing the entire population and decontaminating the animal house facility, followed by replacing them with animals from a pathogen-free source (Lindsey *et al.*, 1991). This paper describes the findings of the salmonellosis outbreak in the colony of Swiss albino mice based on gross necropsy lesions and confirmed by bacteriological isolation, identification and serotyping.

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Materials and Methods

Animals and their Housing

Eighty healthy Swiss albino mice of 8 weeks of age were procured from National Institute of Pharmaceutical Education and Research, Mohali, India and were maintained in the experimental animal house of Department of Veterinary Microbiology, Dr. G.C. Negi College of Veterinary and Animal Sciences, Palampur (H.P., India). The mice were housed in 16 polypropylene cages in group of five per cage. They were fed commercial mice feed @ 5 g/mice/day and had free access to water provided in bottles with metal sipper tubes. Bedding was changed twice weekly. The temperature was maintained at 20-22°C with 40-60% relative humidity and 12 h light/dark cycle in the animal house. The mice were procured for carrying out experiments of the project in the department and were monitored daily for their health status for a period of one month before beginning with the experiments. After 3 week of housing in the animal house some animals showed clinical signs and died unexpectedly. The number of animal showing clinical signs and number of deaths were noted.

Sample Selection and Necropsy

Mice were selected from all the cages and those exhibiting clinical signs were preferentially selected. Cages containing mice with minimal conjunctivitis and diarrhea were selected equally with cages of mice having severe conjunctivitis and diarrhea. The dead animal and those showing clinical signs were submitted for necropsy. The mice showing clinical signs were humanely euthanized in the CO_2 chamber before detailed post-mortem examination. Proper recording of details of gross lesions was done. The representative tissue from the liver, spleen, heart, kidneys, intestine and mesenteric lymph node were collected in 10% neutral buffered formalin for histopathological studies. Fixed tissues were trimmed properly, processed in different grades of alcohol and cleared in benzene and finally embedded in paraffin wax. The sections were cut at 3 to 5 µm thickness and stained by routine haematoxylin and eosin stain (Luna, 1968).

Bacteriological Examination and Serotyping

The tissue and fecal samples collected from mice during necropsy and samples of feed, water and bedding material used in animal house were examined bacteriologically using standard methods for isolation and identification of *Salmonella* (Quinn *et al.,* 2002; ISO, 2018). Briefly, one gram of liver, spleen, kidney, heart, intestinal tissue and fecal sample were homogenized aseptically individually and were streaked on 5% defibrinated sheep blood agar (BA), McConkey Lactose agar (MLA) and Brilliant green agar (BGA) plates and incubated at 37°C for 24 h. One gram of feed, water and bedding material samples were first inoculated in selenite F enrichment broth and then inoculated on BA, MLA and BGA plates. The colonies were characterized morphologically using Gram's staining and were confirmed by conventional biochemical methods (Quinn *et al.,* 2002; Harley and Prescott, 2002). The biochemical tests included oxidase, catalase, indole production, methyl red (MR), Voges-Proskauer (VP), citrate utilization, sulphur reduction, motility, triple sugar iron agar (TSIA), urease, nitrate reduction, lysine decarboxylase and sugar fermentation tests.

The *Salmonella* isolates identified from the tissue and fecal samples of mice were submitted to the Central Research Institute (CRI), Kasauli for serotyping.

Antibiotic Sensitivity Test

The *in vitro* antibiotic sensitivity test of the isolates was conducted on Mueller-Hinton Agar (MHA) using the Kirby-Bauer disc diffusion method in accordance with the standard guidelines of CLSI (CLSI, 2021) using wide range of 10 commonly used antibiotics. Overnight grown bacterial colonies were suspended in 5 mL nutrient broth and incubated at 37°C to obtain the turbidity equivalent to a 0.5 McFarland standard. 100 µL bacterial suspensions spread over the MHA plate and antibiotic discs were placed aseptically on the surface of inoculated medium. Results were recorded within 18-24 h of incubation at 37°C by measuring the diameter of zone of inhibition. The isolate susceptibility was evaluated based on the size of the zones of inhibition and classified as susceptible (S), intermediate susceptible (I), or resistant (R) according to the CLSI criteria.

Results and Discussion

Clinical Signs and Mortality

After three weeks of housing of 80 mice in cages in animal house, 12 mice died and 18 showed clinical signs for over a period of two weeks. After the initial diagnosis of Salmonellosis based on the lesions observed during necropsy of the first five animals that died unexpectedly, all the mice in the colony were monitored for the clinical signs. The clinical signs ranged from dullness, anorexia, ruffled hair coat, hunched posture with varying degree of diarrhea and conjunctivitis as described earlier by Lindsey *et al.* (1991) and Ozkaya *et al.* (2012). However the neurological signs like ataxia and tremors reported by Ozkaya *et al.* (2012) were not evident in the present outbreak. The occurrence of clinical signs may be associated with toxemia produced by the organism once it enters the blood circulation. The lipopolysaccharide (LPS) may be responsible for the endotoxic effects of salmonellae leading to the development of clinical signs (Quinn *et al.*, 2011).

Gross Lesions

In the present outbreak the most significant pathological changes observed were in liver and spleen in addition to finding evidence of endotoxemia, since *S.* Typhimurium was found not only in liver and spleen but also from other organs. The gross pathological lesions of hepatospleenomegaly (Fig.

1) and necrosis in liver and spleen were consistent with the findings of Tunca *et al*. (2012), Shah *et al.* (2013), and Panda *et al*. (2014). Necrosis of the liver included multiple necrotic foci randomly distributed over the liver parenchyma. The hepatic necrosis was characterized by multifocal to coalescing, cream to off white coloured foci of variable size. In addition, moderate liver paleness was visible (Fig. 1). Spleen was moderately congested and swollen. The intestines were filled with mucoid exudates throughout its length along with the foul smelled soft faeces. Mild haemorrhages were present in the mucosa of intestine. The kidneys appeared moderately congested. These gross changes may be probably due to the acute degenerative and inflammatory changes caused by endotoxins produced by the invading organism. Spleenomegaly can be due to the reticular endothelial cell hyperplasia and engorgement of sinuses with blood (Deshmukh *et al*., 2007).

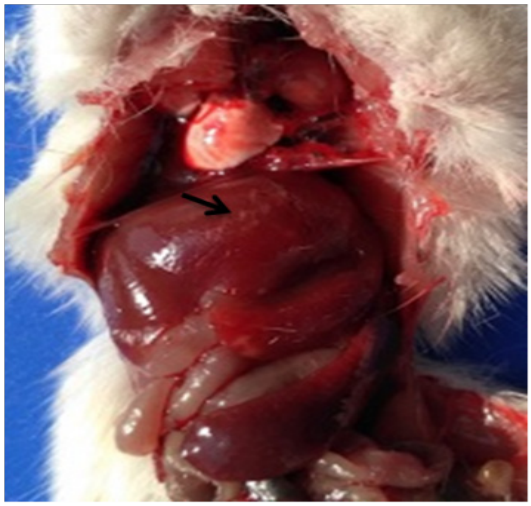


Fig. 1: Liver of mice showing necrotic foci (arrow) of variable size, along with moderate hepatospleenomegaly.

Histopathology

In the liver, focal areas of necrosis along with necrotic area with coalescing pattern were evident throughout the hepatic parenchyma, which were variable in size. The necrotic areas were infiltrated with neutrophils and lymphocytes (Fig. 2a, 2b). Severe sinusoidal dilatation and congestion was evident with multilobed neutrophils. Portal areas around bile ducts were infiltrated with neutrophils, macrophages and lymphocytes (Fig. 2c). The microscopical lesions in the hepatic parenchyma were similar to those described by Deshmukh *et al.* (2005) in the quails infected with the *Salmonella* Gallinarum and the lesions observed in portal areas were in agreement with Neutra *et al.* (1996).

Spleen revealed focal thin areas of necrosis with severe granulocyte infiltration in the splenic parenchyma (Fig. 3a). Haemosiderin pigment was present in plenty. Germinal centers were depleted from lymphocytes in the white pulp and revealed neutrophilic infiltration (Fig. 3b). Mild areas of necrotic foci and hyperplasia were present in the red pulp (Fig. 3c) and arteritis was evident with infiltration of neutrophils and lymphocytes. Similar microscopic lesions in the spleen were also reported previously by Mamta *et al.* (2010), Haider *et al.* (2012), Tunca *et al.* (2012), and Panda *et al.* (2014) in their studies.

The intestine revealed severely congested blood vessels with mild neutrophilic infiltration in lymphoid element along with moderate depletion of lymphocytes. In intestine the long cylindrical villi were present with congested corium and apical region of villi. Severe goblet hyperplasia was evident along with the mucus exudates in lumen of intestine which was in agreement with the earlier studies of Arnold *et al.* (1993).

In heart, the muscle fibers were infiltrated with mononuclear cells (Fig. 4). In kidney mononuclear cell infiltration was observed in interstitial tubules and epithelial cells of proximal convoluted tubules appeared slightly

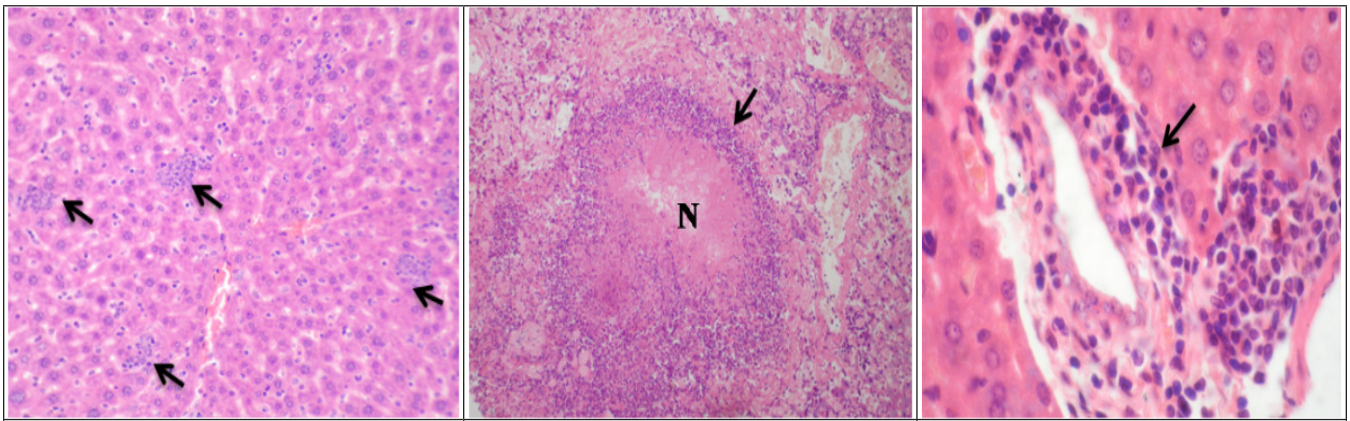
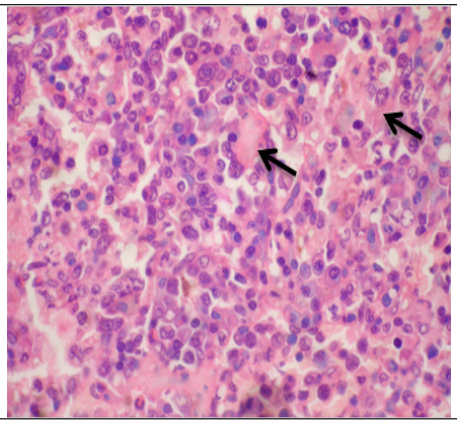


Fig. 2a	Fig. 2b	Fig. 2c

Fig. 2: Photomicrographs of the liver of mice stained with Haematoxylin & Eosin (H&E). **2a**. Multifocal areas of necrosis (arrows) infiltrated with neutrophils and lymphocytes, H&E X 132. **2b**. Coalescing pattern of necrosis (N) surrounded by degenerated neutrophils, lymphocytes and fibroblast (arrow), H &E X 66. **2c**. Mononuclear cells infiltration (arrow) around the bile duct, H &E X 330.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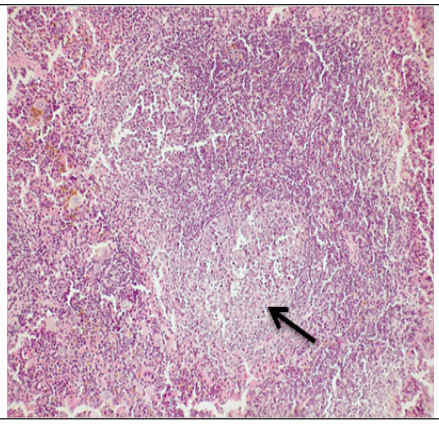
Fig. 3b

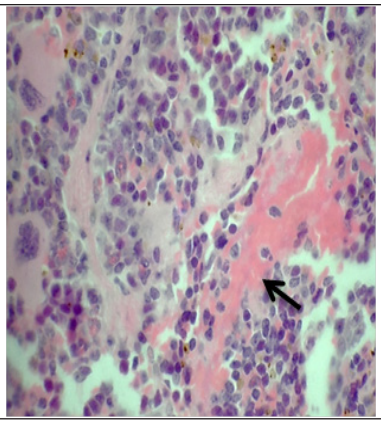
Fig. 3c

Fig. 3: Photomicrographs of the spleen of mice stained with Haematoxylin & Eosin (H&E). **3a.** Focal thin area of necrosis (arrows) and increase in the number of granulocytes in the parenchyma, H&E X 132. **3b.** Depletion of lymphocytes in the germinal centre (arrow) of the white pulp, H&E X 66. **3c.** Homogenous eosinophilic area of necrosis (arrow) in the red pulp, H &E X 330.

granular. Perivascular and peritubular infiltration of lymphocytes were also evident.

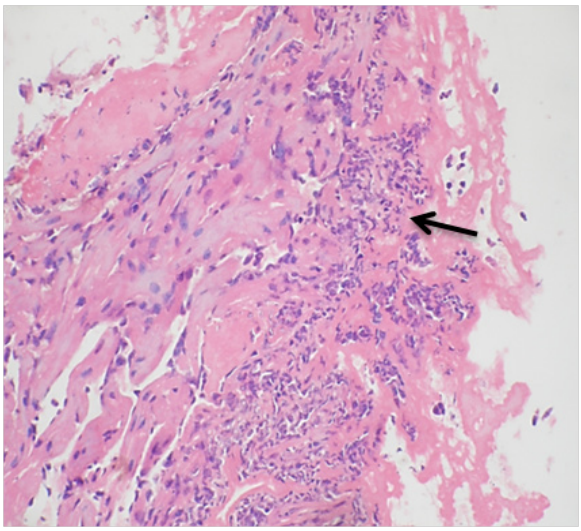

Fig. 4: Photomicrograph of the heart of mice showing mononuclear cell infiltration (arrow) in the myocardium, H&E X 66.

Bacteriological Findings

The growth on BA plate was indicated by circular, smooth, opaque, translucent colonies with heamolysis. On BGA plate, the colonies were pale pink color against a pinkish background. In the case of MLA plates, smooth yellow colonies were observed, while on TSI agar slant there were black coloured colonies. Gram's staining revealed Gram-negative, pink colored, small rods, arranged in single or in pairs. The isolates were found to be motile having swinging movement when examined using hanging drop slide under microscope. The isolates gave positive result for catalase, methyl red, citrate utilization, sulphur reduction, nitrate reduction and lysine decarboxylation test and negative result for oxidase, indole production, Voges Proskaur and urease test. The isolates fermented dextrose, maltose and mannitol with the production of acid and gas, but did not ferment lactose and sucrose. The bacteriological examination confirmed the presence of *Salmonella* Typhimurium which was in agreement with the findings of Gene (2002), Sogard *et al.* (2007) and Han *et al.* (2011).

To trace the source of outbreak feed, water and bedding material used in the animal house were examined for contamination with *Salmonella*. All samples were found negative for *Salmonella*. The source of Salmonella infection which caused the outbreak in the mice colony was not traced. However, 10 days before the onset of disease the laboratory animal unit staff member reported presence of wild rodent in the laboratory animal house that was suspected as source of infection.

Serotyping

The isolates from the necropsy and fecal samples were serotyped from Central Research Institute, Kasauli and were identified as *Salmonella enterica* serotype Typhimurium 4, 12:i:1, 2.

Antibiotic Sensitivity Test

The isolates were susceptible to amoxyclav, ciprofloxacin, norfloxacin and gatifloxacin but intermediate susceptible to cefuroxime, ceftriaxone, cephotaxime and cefamendole. The organism was resistant to streptomycin and gentamicin (Table 1). However, the treatment of animals with antibiotics was not carried out since the antibiotics may serve to treat illness, but, the problems of carrier state always remain in salmonellosis. The rodents infected with *Salmonella* are zoonotic risk to personnel, a source of infection to other animals in the facility and unsuitable for research purposes (Lindsey *et al*., 1991). Therefore, the mice were not used for the research experiments and all the mice in the colony were humanely euthanized in the CO_2 chamber and the animal house was decontaminated.

Table 1: Antibiotic sensitivity test of *Salmonella enterica* serotype Typhimurium on disc diffusion test.

Antibiotic	Zone of Inhibition (mm)	Interpretation
Amoxyclav	21	Susceptible
Cefamendole	16	Intermediate
Ceftriaxone	21	Intermediate
Cefuroxime	17	Intermediate
Cephotaxime	18	Intermediate
Ciprofloxacin	22	Susceptible
Gatifloxacin	19	Susceptible
Gentamicin	8	Resistant
Norfloxacin	18	Susceptible
Streptomycin	0	Resistant

Conclusion

The outbreak of *Salmonella enterica* serotype Typhimurium 4, 12:i:1, 2 was reported in the colony of Swiss albino mice. This organism is highly pathogenic which produces severe systemic infection in mice with lesions mainly in the liver, spleen and intestine of mice. From our experience of disease outbreak we inferred that routine screening of laboratory animals and gross necropsy examination is helpful in early diagnosis of disease since the first indication of an unwanted organism's presence came from clinical cases.

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