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Pathomorphological Changes in Various Organs of Experimentally Induced *Salmonellosis* in Mice

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ABSTRACT

In the study, a total of 36 BALB/C mice divided into twelve equal groups were used. Eleven groups were administered 50 µl of inocula containing 10^7 CFU prepared from eleven isolates of *Salmonella* Typhimurium, administering one isolate in each group. One group was kept as negative control and each mouse in this group was given 0.5 ml of normal saline per os.The study was conducted for 15 days during which symptoms *viz.*, ruffled feathers, hunched posture, etc., exhibited by the mice were observed and deaths were recorded. At necropsy, gross lesions of moderate to severe focal hepatic necrosis characteristic of *Salmonella* infection were observed. In some cases, brain and spleen were congested. No significant gross lesions were observed in heart, lung and kidney. *Salmonella* was isolated from liver, spleen and brain of mice that died1st to 4th day post- exposure. Surviving mice were sacrificed on 14th day post- exposure, three of these had necrotic foci on the liver and *Salmonella* was isolated from all of these mice.

Keywords*:* *Salmonella,* mice, pathogenicity.

Salmonellosis is a direct occupational anthropozoonotic disease of great economic and public health concern. Salmonellae are widely distributed in nature and cause a spectrum of diseases in man, animals and birds. Poultry eggs, meat and their products are the commonest vehicles of *Salmonella* to humans. In India, Salmonellosis is hyper-endemic and there is an urgent need to strengthen the monitoring and surveillance of salmonellosis using suitable diagnostic tools so as to prevent and control its occurrence (Rahman, 2002). Of the 2435 reported serovars of *Salmonella*, 209 have been reported from India and *Salmonella* Typhimurium being the most common serovar prevalent both in man and animals (Verma *et al.*, 2001).

The genus *Salmonella* is a member of the family *Enterobacteriaceae* and consists of Gram -ve, oxidase -ve, straight sided rod shaped bacteria which are castalase +ve, and have both a respiratory and a fermentative metabolism of carbohydrates. *Salmonella* infection arising from contaminated food continues to be an immense problem with millions of cases occurring annually throughout the world, detection of *Salmonella* before contaminated foods can be consumed is therefore an essential feature of safeguarding public health. Surveillance of *Salmonella* in all the different stages of food chain constitutes an important element in the exploration of epidemiology of food borne salmonellosis, and in the development and implementation of efficient *Salmonella* control strategies. *Salmonella* serotypes have a broad host range and clinical manifestations that result from the combination between serotype and host species involved. The mouse, which has been used for most of the studies addressing the various aspects of host-pathogen interaction in *S.* Typhimurium infection, develops a systemic disease when infected with *S.*, Typhimurium but no diarrhea. Studies using mice and other animal models of *Salmonella* diseases have yielded substantial data about the molecular players involved at different levels.

MATERIALS AND METHODS

Experimental animals

Apparently healthy BALB/C mice were procured from Indian Institute of Integrative Medicine (CSIR Lab), Jammu and randomly divided into twelve groups (group-I, to XII) having three mice in each group. The animals were housed in under specific pathogen free conditions in polypropylene cages with free access to sterilized pelleted feed and water. A daily cycle of 12 h of light and similar 12 h of darkness was provided to animals. Approval from Institutional Animal Ethics Committee was sought to conduct the experimentation.

Pathogenicity of *Salmonella* infection

Pathogenicity of *Salmonella* infection in mice was studied as per method of Barthel *et al*. (2003). *Salmonella* Typhimurium strains were grown for 12 hrs at 37 °C in Brain Heart Infusion broth (Himedia, M210). Bacteria were washed twice in phosphate buffer saline (PBS) and then suspended in PBS and the inoculum was adjusted to 10^7 CFU/50 µl. After 72 hours of acclimatization, mice in the group I to XI were administered 50 µl of inocula prepared from eleven isolates, administering one isolate in each mouse of the eleven groups. Group XII was kept as negative control and each mouse of this group was given 0.5 ml of normal saline per os. The experiment was carried out for 15 days during which symptoms *viz.*, ruffled feathers, hunched posture, etc., exhibited by the mice were observed and deaths were recorded.

Histopathological changes

Postmortem of the dead mice was carried out and gross and histopathological changes were noted in liver, spleen, kidney, brain, heart, and lungs. Cultural examination of organs was also done. At the end of the trial, the surviving animals were sacrificed by cervical dislocation and their organs were collected for histopathological examination. For histopathology, the organs were fixed in 10% (v/v) neutral buffered formalin solution. After 3-4 days of fixation, tissues were washed in running water for 7-8 hrs, dehydrated in ascending grades of ethyl alcohol, cleared in benzene and embedded with melted paraffin wax (melting point 58^0C). The paraffin blocks were prepared and sections were cut at 4-5µ thickness with a hand operated microtome. The paraffin embedded sections were then passed through sequential steps of de-paraffinisation in xylene, rehydration by passing through descending grades of ethyl alcohol to running tap water and stained with routine haematoxylin and eosin stain (H&E) as per method of Luna (1968).

RESULTS AND DISCUSSION

The mice were grouped into from I to XII groups. Twenty four hours after administration of inocula, some mice in each group, except mice in group XII, were lethargic and withdrawn. Few appeared severely ill and deaths occurred between 1st to 4th day post- exposure. In group I, IV, V, IX, X and XI one death each was recorded, whereas no death was recorded in group II, III, VI, VII, VIII as well as negative control.

At necropsy, gross lesions of moderate to severe focal hepatic necrosis characteristic of salmonella infection were observed. In some cases, brain and spleen were congested. No significant gross lesions were observed in heart, lung and kidney. Salmonella was isolated from liver, spleen and brain of mice that died1st to 4th day post-exposure. Surviving mice were sacrificed on 14th day post-exposure, three of these had necrotic foci on the liver and *Salmonella* was isolated from all of these mice.

Histopathological examination revealed more severe changes in liver and included necrotic foci with vacuolar hepatocytic degeneration, individualization, loss of cord pattern and mild infiltration mononuclear cell early salmonella lesion (Fig. 1). Similar change with presence of microabscesses containing necrotic cell debries and polymorphonuclear cells surrounded by necrotic hepatocytes and typhoid granuloma were also observed in 3 mice livers (Fig. 2). Our findings agree with those reported by Mastroeni *et al.* (1995) who studied the histopathology of salmonella infection in mice by inoculating the suspension of the virulent strain *Salmonella* Typhimurium C5 and *S.* Typhimurium M525, (a strain with intermediate virulence) into the lateral tail vein of animals and observed the persistence of well-defined granulomatous lesions, invaded from the periphery to the center by mononuclear cells, in the liver and spleen of mice in the earlier stage while the lesions observed later on contained more lymphocytes. Sundari *et al.* (2011) have

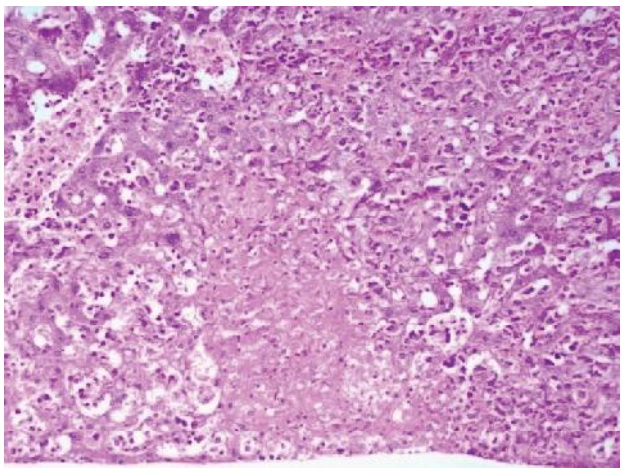

Fig. 1: Necrotic foci in liver vacuolar hepatocytic degeneration, individualization, loss of hepatic cord pattern, mild infiltration mononuclear cell early salmonella lesion H&E X200.

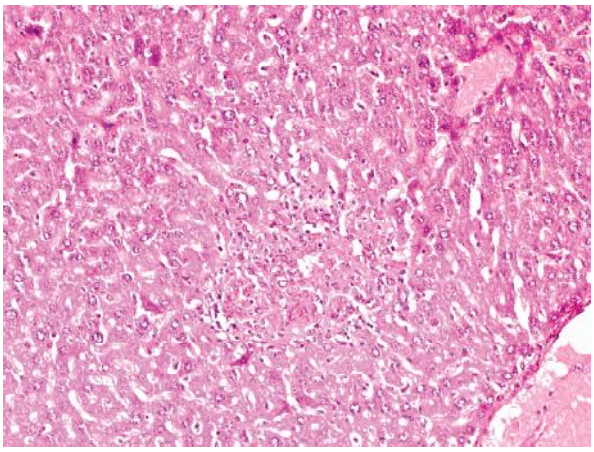

Fig. 2: Focal vacuolar degeneration of hepatocytes with loss of cord pattern and microabscesses containing necrotic cell debris and polymorphonuclear cells, single typhoid granuloma H&E X200.

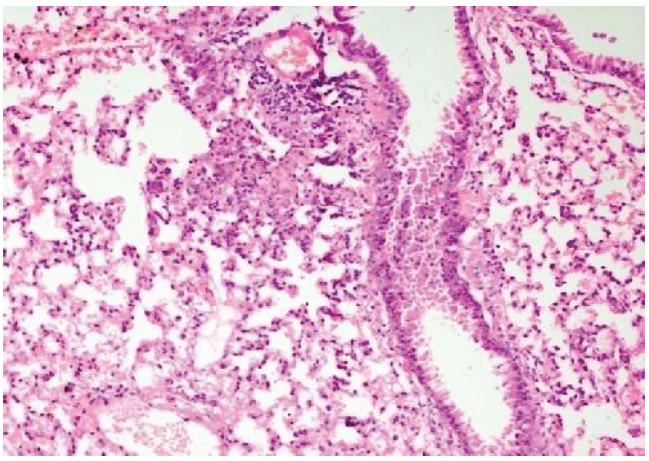

Fig. 3: Section of lung showing mild interstitial pneumonia and cellular debris in a bronchiole. H&E X200.

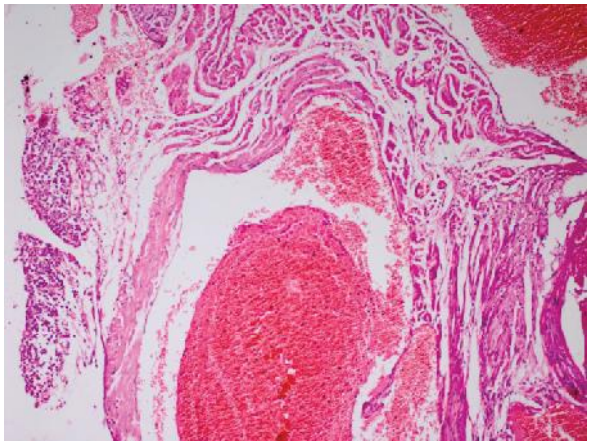

Fig. 4: Section of heart showing epicarditis, engorgement of intermyocardial blood vessel and thinning of intermyocardial fibres H&E X100.

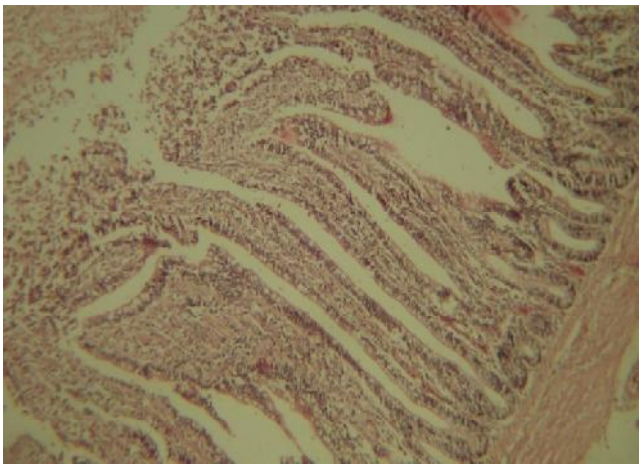

Fig.5. Section of intestine showing extensive necrosis sloughing of enterocytes of villi, cellular infiltration in lamina properia and goblet cell hyperplasia. H&E X100.

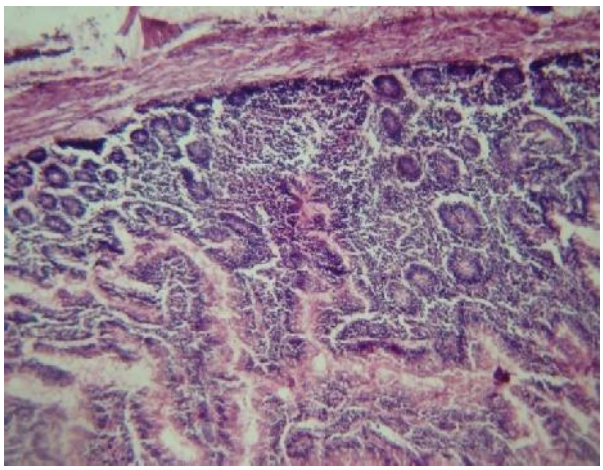

Fig. 6: Peyer,s patches showing degeneration and depletion of lymphocytes leaving empty space. H&E X100

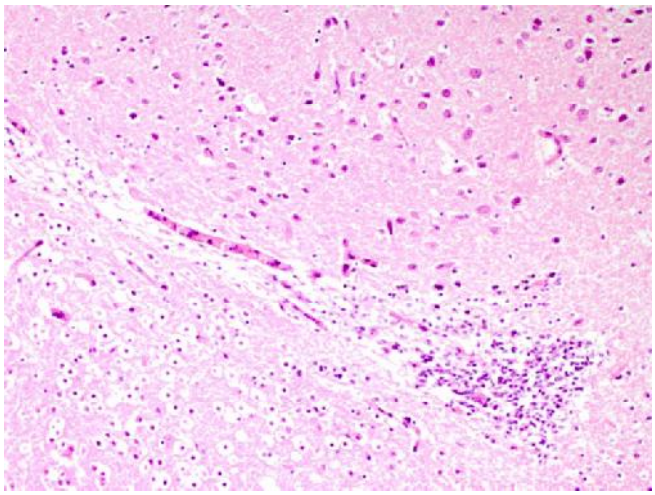

Fig. 7: Section of brain showing focal area of gliosis and blood vessel engorgement H&E X100

reported similar findings in liver, intestines and spleen of mice, experimentally infected with *Salmonella*.

Section of kidneys of infected mice revealed mild degeneration and swelling of epithelial cells of PCTs and interstitial haemorrhages. Some section showed more severe damage including necrosis of tubular epithelial cells. Focal area of necrosis infiltrated by MNC and macrophages were also observed in two mice. In another study, Yehya and Salman (2008) have reported degenerative changes in renal tubules of kidney of mice injected subcutaneously with 5x106 ^{CFU} of *Salmonella* Enteritidis. Their results nearly agree with our findings.

Lungs showed the focal haemorrhages in interstitial spaces and edematous fluid in alveoli in sacrificed mice. However, more severe changes were observed in dead mice. Mild interstitial pneumonia, haemorrhage and cellular debris in bronchiole were observed in two dead mice (Fig. 3). In a similar study, however on *Salmonella* Paratyphi, by Al-Joboury (2007), a high inoculum of *Salmonella* Paratyphi (8 x 10^8 CFU) administered intraperitoneally in mice reportedly produced congestion and mild interstitial pneumonic lesions in lungs nearly similar to those observed in our study. Use of high inoculum (8 x 10^8 CFU of *Salmonella* Paratyphi) was justified since *Salmonella* Paratyphi is strictly pathogenic for humans (or higher primates) but not naturally virulent for mice.

Heart showed areas of epicarditis and engorgement of intermyocardial blood vessels and thinning of intermyocardial fibres (Fig. 4) in sacrificed mice. More severe changes such as degeneration and necrosis of myocardial fibres diffusely infiltrated by MNCs were seen in dead mices. Yehya and Salman (2008) have reported similiar findings in mice experimentally infected with *Salmonella* Enteritidis wherein they observed hemorrhages and edema in between cardiac muscle with areas of slight destruction and infiltration by mononuclear cells.

Section of spleen showed the red pulp congestion and haemosiderosis in three mices sacrificed. However, mices died earlier showed the severe congestion of red pulp and depletion of lymphoid cells leaving the clear empty spaces and even atrophy of splenic follicles were also observed in some mices. Our results were in corroboration with other workers (Sundari *et al.*, 2011; Nakoneczna and Hsu, 1980).

Intestine of two sacrified mice showed blunting and fusion of villi, hyperplasia of goblet cells and submucosal blood vessel engorgement. In other 3 mice, cellular infiltration in lamina propria and goblet cell hyperplasia and degeneration and necrosis of enterocytes leaving necrotic remnants (Fig. 5). The Peyer's patches showed changes degeneration and necrosis of lymphocytes leaving empty space (Fig. 6). In a similar study, Barthel *et al.* (2003) observed more severe changes in the intestines of mice experimentally infected with *S.* Typhimurium. The reason could be that the mice used in their study were pre-treated with streptomycin. The treatment with streptomycin might have reduced the oral infectious dose of serovar Enteritidis or serovar

Typhimurium and greatly improved intestinal colonization due to the elimination of commensal intestinal bacteria.

Histological changes observed in the brain included marked leptomeningeal congestion, edema, and infiltration of lymphomononuclear cells, perivascular mononuclear cell infiltration and increased perivascular space (Fig. 9) and focal area of gliosis and blood vessel engorgement in infected mice. Our findings are in agreement with those of Wickham *et al.* (2007) who in their study, after oral infection of mice with *Salmonella* Typhimurium, found the presence of patchy meningitis confined to the sub-arachnoid space in mice with neurological deficit.

CONCLUSIONS

Salmonella isolates were orally administered to the mice to evaluate the pathomorphological effects in various organs of the infected mice. Prominent gross lesions included pin point haemorrhages on liver, congestion and grey hepatization of lungs and congestion in kidneys, brain and spleen. Histopathologically, more severe changes were observed in liver that included vacuolar hepatocytic degeneration, individualization, loss of hepatic cord pattern, multiple areas of centrilobular hepatocytic necrosis and formation of focal necrotic granuloma infiltrated by MNC's. In kidneys mild degeneration and swelling of epithelial cells of PCT's with necrosis and interstitial haemorrhages was observed. Heart showed areas of epicarditis and engorgement of intermyocardial blood vessels, thinning of intermyocardial fibres, degeneration and necrosis of myocardial fibres diffusely infiltrated by MNCs. Lungs revealed the focal haemorrhages in interstitial spaces, edematous fluid in alveoli and interstitial pneumonia. The spleen showed red pulp congestion, haemosiderosis, depletion of lymphoid cells leaving the clear empty spaces and even atrophy of splenic follicles whereas in intestines blunting and fusion of villi, degeneration and necrosis of enterocytes, hyperplasia of goblet cells and submucosal blood vessel engorgement was seen.

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