PATHOGENESIS STUDY OF PORCINE SALMONELLOSIS IN MOUSE MODEL

C. Prasad[1], Ajith Jacob George[2], N. Divakaran[3], K. Krithiga[4] and Binu K Mani[5]
Department of Veterinary Pathology,
College of Veterinary and Animal Sciences,
Mannuthy-680651, Thrissur, Kerala.

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Abstract

A study was undertaken to isolate the Salmonella serovars from gastroenteritic lesions of piglets and to assess the pathogenic effect of porcine Salmonella in gastrointestinal tract of mice. Tissue samples were collected from piglets showing varying degrees of gastroenteritis. The tissue samples were subjected to different conventional culture methods and biochemical tests for Salmonella serovar identification. Ten sample isolates were isolated from 90 samples and were identified as Salmonella Choleraesuis. *The major virulent Salmonella isolate with infective dose of 2.7 x 10^7 CFU/ml was administered orally for pathogenesis study to 48 mice based on the pilot study along with a control group of six mice. Six mice each were sacrificed by cervical dislocation at 6, 12, 24, 36, 48, 60, 72 and 96 h, post-inoculation. Detailed postmortem examination was conducted and gross lesions particularly of gastrointestinal tract (GIT) were recorded. Gross lesions of mice revealed severe catarrhal enteritis, hepatic degeneration and pulmonary emphysema at varying intervals. The representative tissue samples of gastrointestinal tract were collected in 10 per cent neutral buffered formalin and processed for histopathological examination.*

Keywords: *Piglets, Salmonella, Pathogenesis study, Mice*

Salmonella is considered as ubiquitous and natural commensal bacteria found in the GIT of both animals and human beings. The *Salmonella* gets activated during unfavorable conditions like post weaning period, infectious disease, parasitic infection, contaminated feed and environmental and poor managemental practices. *Salmonella* spreads to human beings through consumption of infected pork which imparts major health concern. Salmonellosis in piglets was the most commonly encountered gastrointestinal disease and the diseased piglets acts as natural reservoir for the spread of the infection. *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Choleraesuis plays lead role in causing salmonellosis in pigs. The present study was mainly envisaged to isolate the *Salmonella* from gastroenteritic lesions of pigs and to study its pathogenic effect on GIT of mice.

1. M.V.Sc. Scholar
2. Associate Professor, Email id: ajithjacob@kvasu.ac.in
3. Professor and Head.
4. Assistant Professor
5. Assistant Professor, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy.

Materials and Methods

A sum of 90 samples were collected at random from ileum, caecum and colon of 90 piglets brought for autopsy to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy which were characterized grossly by marked lesions in the GIT. Under aseptic conditions, the dorsum of the sample was cleaned using sterile cotton dipped in alcohol. The surface was then seared using a hot spatula and an incision was made superficially at its center using a sterile scissor. A sterile bacteriological inoculation loop was inserted through the incision, rotated inside the tissue, taken back and inoculated in the media. Isolation of bacteria associated with the lesion was done by culturing on MacConkey Agar (MA), Brilliant Green Agar (BGA) and Brain heart infusion agar (BHIA).

The *Salmonella* suspected colonies were subjected to different biochemical tests that include indole test, methyl red test (MR) , voges-proskaur test (VP), citrate test, urease test, catalase test, oxidase test and triple sugar iron test (TSI). The isolates that showed positive in the above tests were further subjected to different sugar fermentation tests viz., mannitol, ionositol, sorbitol, trehalose, maltose, dulcitol, rhamnose and ortho-nitro phenyl beta-D-galactopyranoside test (ONPG). Based on the biochemical and sugar fermentation test, the *Salmonella* serovar differentiation was done.

The major virulent *Salmonella* isolate obtained was selected for the pilot study. The selected isolates were inoculated into Brain Heart Infusion Broth (BHIB) and incubated at 37 ºC overnight. The optical density (OD) of the culture broth was measured at 600 nm using spectrophotometer. The obtained value was compared with standard table for infective dose assessment. Dose of 2.7 x 10^6 CFU/ml, 2.7 x 10^7 CFU/ml and 2.7 x 10^8 CFU/ml (Sundari *et al*., 2011) were administered orally into three groups of five BALB/C mice each. The group which showed maximum survival with clinical symptoms up to 96 hours was determined. This dose was used for the experimental study.

Fifty-four adult BALB/C mice of approximately six to eight weeks of age procured from Small Animal Breeding Station, Mannuthy, were used for the present study. All the mice were maintained in well ventilated cages on normal standard ration. The virulent *Salmonella* isolate obtained was administered orally at an infective dose of 2.7x10^7 CFU/ml to 48 mice. A control group of six mice were maintained without any treatment. They were examined closely for clinical symptoms. Six mice each were sacrificed at 6, 12, 24, 36, 48, 60, 72 and 96 h, post-inoculation (T1 to T-8). Detailed post-mortem examination was conducted and gross lesions particularly of GIT were recorded. Tissue samples were collected in 10 per cent neutral buffered formalin for histopathological studies using standard protocols (Bancroft and Gamble, 2007).

Results and Discussion

Ten isolates of *Salmonella* Choleraesuis were isolated and identified from 90 intestine samples that showed gastroenteritic lesion from different piglet carcasses. On post mortem examination of the piglet carcasses, GIT revealed catarrhal to diphtheritic enteritis. Moderate to diffuse, yellow to cream colored diphtheritic membrane formation, fibrino-necrotic deposits and varying degrees of ulcers; button and erosive ulcers, were more predominant in caecum and colon. The lesions in other organs were multifocal white patchy areas of necrosis in liver, severe consolidation of lungs, moderate enlargement and petechial hemorrhages in spleen, and pale discolouration of kidneys. Reed *et al*. (1986) reported that the experimental oral infection of *Salmonella* Choleraesuis in pigs resulted in interstitial pneumonia, septicemia, multifocal necrosis in liver, massive ulceration and necrotic colitis. Perez *et al.* (1999) reported that gross lesions of *Salmonella* Choleraesuis infection in pigs were characterized by multifocal yellow colored foci on liver with mild enlargement, splenomegaly, mesenteric lymphadenopathy with sub-capsular and medullary hemorrhage, diffuse consolidation of cranial and caudal lobe of lungs, petechial hemorrhage in the endocardium, cortex of kidney, and serosal layer of intestine, circular to linear ulcerated areas in ileum, caecum and

colon with presence of fibrino necrotic material in the lumen of the large intestine. Ecco *et al.* (2006) reported that *Salmonella* Choleraesuis infection in pigs was associated with presence of tubular cast of friable yellow necrotic material in large intestinal lumen and diffuse diphtheritic fibrino necrotic typhlitis and colitis. The present study also revealed similar gross lesions that were consistent with the previous reports of salmonellosis in pigs.

Ten *Salmonella* isolates were obtained from the gastroenteritic intestinal samples from 90 different piglet carcasses. The isolates were characterized by gram negative, rod shaped and immotile organisms. They gave non lactose fermenting colorless colonies in MA and pink colored colonies in BGA (Fig. 1). The serovar differentiation was done based on the IMViC, sugar fermentation test, TSI, catalase test and oxidase test. Indole (-), MR (+), VP (-), citrate test (+), TSI (yellow red), urease (+), catalase (+), oxidase (-), inositol (-), sorbitol (+), trehalose (-), maltose (+), ONPG (-), dulcitol (-), rhamnose (+) and mannitol (+) were observed (Fig. 2 & 3). Biochemically, the *Salmonella* isolates were identified as *Salmonella* Choleraesuis. Molla *et al.* (2006) reported similar method of isolation of *Salmonella* from liver and caecal contents of pigs, based on conventional culture methods and biochemical tests. Irimie *et al.* (2010) isolated *Salmonella* Choleraesui*s* from liver and gastroenteritic lesions of weaned piglets by streaking in BGA. He also identified the serovar by performing various biochemical tests and sugar fermentation tests. Sakano *et al.* (2011) reported the isolation of *Salmonella* from liver, mesenteric lymph node and lung of pigs by using BGA and different biochemical tests and identified as *Salmonella* Choleraesuis. From these reports, it can be concluded that *Salmonella* Choleraesuis can be isolated from the gastroenteric lesions and could be identified using conventional methods.

On close examination of the *Salmonella* infected mice in pilot study, there was anorexia, lethargy, ruffled hair coat and restricted movement. Malik *et al.* (2015) noticed lethargy after 24 h post infection (PI) with *Salmonella*. In the present study, mice had lethargy and anorexia after 24 h PI. Gross lesions revealed hepatosis, catarrhal enteritis, mild splenomegaly, pulmonary congestion and emphysema. The lesions were similar to that of pig salmonellosis.

Experimental infection (T1-T8) of *Salmonella* Choleraesuis in mice revealed extreme distension of the stomach, mild congestion of blood vessels and moderate to severe catarrhal enteritis was observed in intestine from 12 h PI (Fig. 4). No other gross lesions were noted. The stomach and intestines of control group showed apparently normal appearance. Liver showed slight pale discolouration from 6 h PI. From 12 h PI, there was gradual increase in the paleness of the liver and evident for necrosis up to 96 h. At 48 h PI, necrosis in liver was predominant. Focal to multifocal small white spots were evident in liver from 36 h to 96 h PI (Fig.5). The liver of controls animal were apparently normal and no detectable gross lesions were present. Lungs, heart and spleen appeared normal in all groups of animals. Kidney showed mild hemorrhage in the cortical surface in all the groups. Our findings agree with those reported by Sundari *et al.* (2011) who reported splenomegaly, congestion in liver, lungs, heart and intestines filled with watery fluid in mice orally administered with *Salmonella*. Malik *et al.* (2015) observed moderate to severe focal hepatic necrosis in the experimentally infected mice at day 14 PI.

Histopathological lesions in GIT of *Salmonella* infected mice revealed mild mononuclear cell infiltration in sub-mucosa and erosion at tip of villi of stomach at 6 h and 12 h post infection (PI) (Fig. 6 & 7). Duodenum showed mononuclear cell infiltration in sub-mucosa and villous fusion at 48 h PI, mild villous fusion and erosion at tip of villi at 60 h PI and moderate mononuclear cell infiltration in sub-mucosa at72 h PI (Fig. 8-10). Jejunum showed mild mononuclear and neutrophil infiltration with edema in the sub-mucosa at 24 h PI (Fig. 11). Marked hypertrophy of goblet cells was noticed in ileum at 6 h PI (Fig. 12). Colon showed severe goblet cell hyperplasia at 6 h PI (Fig.13). Malik *et al.* (2015) also reported similar lesions in intestines characterized by blunting and fusion of villi, hyperplasia of goblet cells, congestion in sub-mucosa and mononuclear

Fig. 1. Colonies of *Salmonella spp* with characteristic pink colour in BGA agar

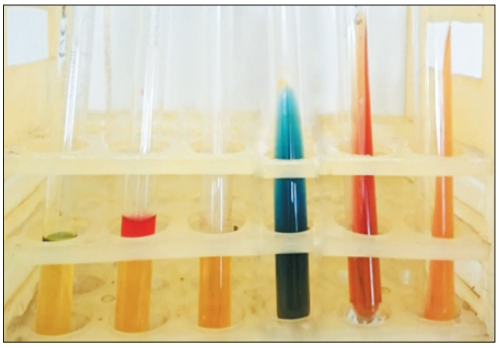

Fig. 2. IMViC, TSI and Urease test for *Salmonella spp.* (I-Indole; MR- Methyl red; VP-Voges Proskaur; C-Citrate; TSI-Triple sugar iron)

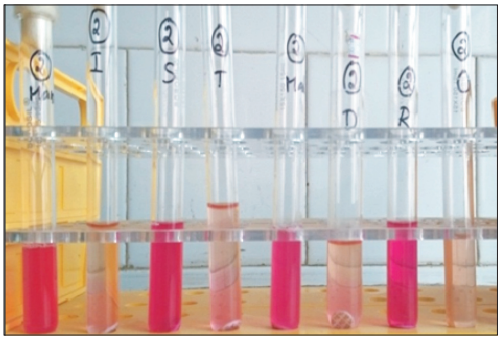

Fig. 3. Different sugar test for *Salmonella spp.* (Mannose; Ionositol; Sorbital; Trehalose; Maltose; Dulcitol; Rhamnose and ONPG

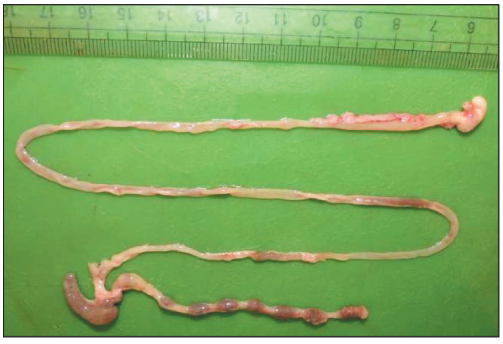

Fig. 4. GI tract - Moderate catarrhal enteritis - at 48 h PI

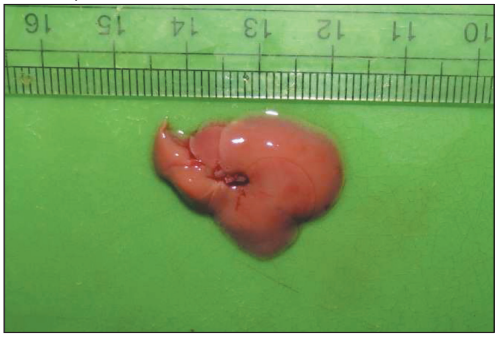

Fig. 5. Liver - Moderate pale discolouration - at 96 h PI

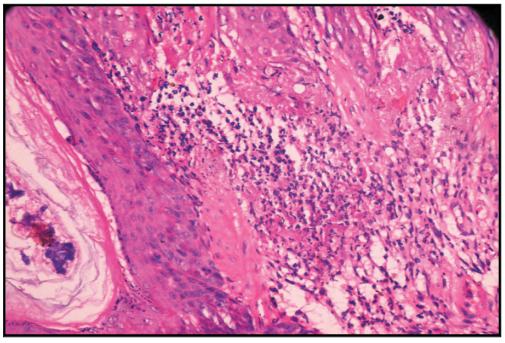

Fig. 6. Stomach – Mild mononuclear cell infiltration in sub-mucosa – at 6 h PI - H&E x 400

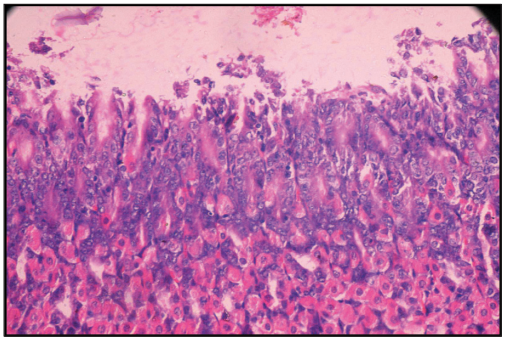

Fig.7. Stomach – Mild Erosion at tip of villi – at 12 PI - H&E x 400

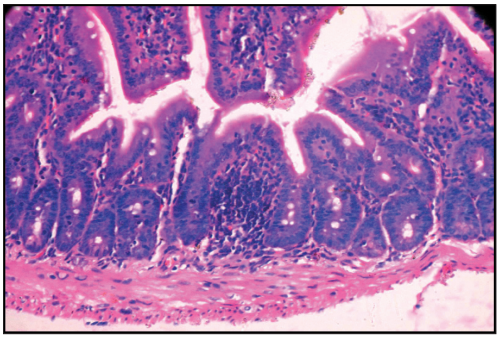

Fig.8. Duodenum - Mononuclear cell infiltration in sub-mucosa and villous fusion - at 48 PI - H&E x 100

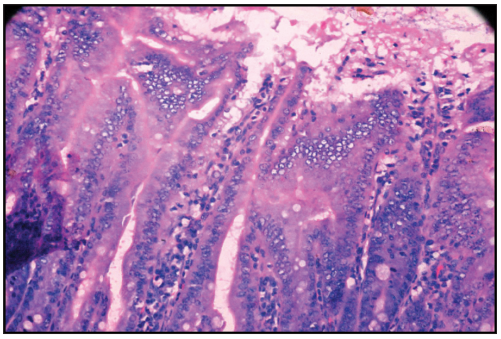

Fig.9. Duodenum – Mild Villous fusion and erosion at tip of villi – at 60 PI - H&E x 400

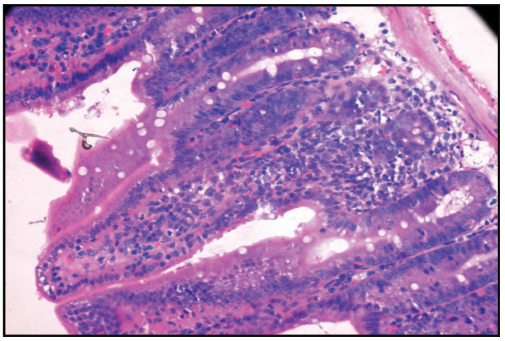

Fig.10. Duodenum –Moderate mononuclear cell infiltration in sub-mucosa – at 72 PI - H&E x 400

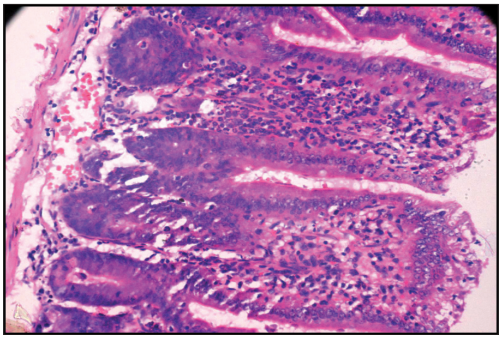

Fig.11. Jejunum – Mild mononuclear and neutrophil infiltration with edema in the sub mucosa – at 24 PI - H&E x 400

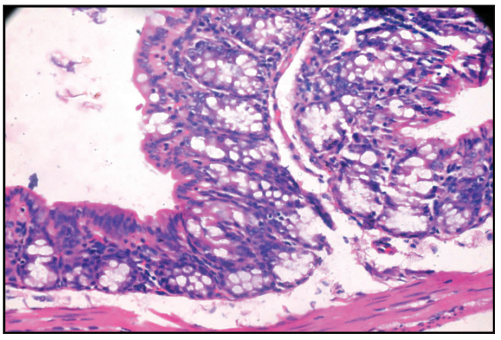

Fig.12. Ileum – Marked hypertrophy of goblet cells – at 6 PI - H&E x 400

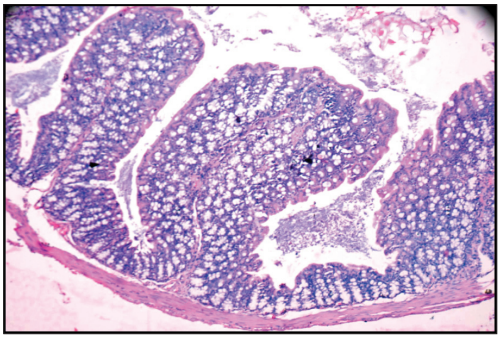

Fig.13. Colon – Severe goblet cell hyperplasia - at 6h PI - H&E x 100

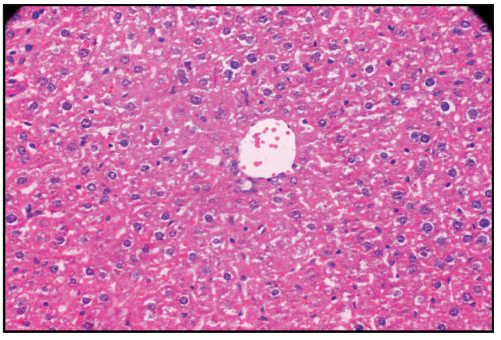

Fig.14. Liver – Diffuse cloudy swelling of hepatocyte and centrilobular necrosis – at 24 h PI – H&E x 400

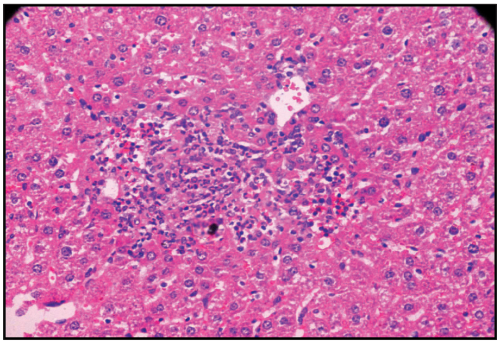

Fig.15. Liver – Micro granuloma that contain both lymphocytic and neutrophilic infiltration -at 24 PI – H&E x 400

cell infiltration in the lamina propria layer in the experimental salmonellosis of mice. Sundari *et al.* (2011) reported villous destruction and mucosal congestion with presence of lymphoid cell aggregation in the intestine of *Salmonella* infected mice. Histopathological lesions in the present study were correlated with Sundari *et al.* (2011) and Malik *et al.* (2015).

Liver showed micro granuloma formation with diffuse infiltration of both neutrophil and lymphocytes. Diffuse cloudy

swelling of hepatocytes and centrilobular necrosis at 24 h PI (Fig.14 & 15). Malik *et al.* (2015) also reported micro granuloma formation with mononuclear cell infiltration and severe vacuolar degeneration in liver; goblet cell hyperplasia, atrophy and fusion of villi and sub-mucosal congestion in the intestines of *Salmonella* infected mice 1st to 4th day PI. No characterized lesions were observed in lungs, heart and spleen. *Salmonella* was re-isolated from the intestinal samples of mice by streaking in MA, BGA and confirmed by biochemical test (IMViC). Sundari *et al.* (2011) also re-isolated *Salmonella* from the fecal samples using conventional methods.

The present study revealed that experiment *Salmonella* Choleraesuis infection in mice produced mild to moderate lesions in the stomach, intestines, liver and kidney. Previous study done in the department has found Salmonellaa associated with gastroenteritis in pigs. In the present study, the most prevalent serovar was identified and characterized. The study also identified mice as ideal mode for study of progressive pathogenesis of the disease. If the infection persisted for long period, lesions would have aggravated and produced a serious gastrointestinal disease. The findings from the above study were similar to previous reports of experimental salmonellosis in mice.

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