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Pathological Studies and Molecular Detection of Salmonellosis in Desi Chickens of Nagpur Region

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ABSTRACT

Avian salmonellosis is an important disease causing serious impediment to the development of poultry industry especially in developing countries. During present study, mortality due to Salmonellosis in six desi chicken flocks belonging to Nagpur region of Maharashtra was noticed. Characteristic lesions of necrotic foci on liver, omphalitis and catarrhal enteritis were noticed in *Salmonella* spp. affected chickens. Colourless colonies on MLA, light pink colonies on BGA and jet black colonies on XLD agar confirmed the *Salmonella* spp. infection. Clinical samples of liver collected from six desi chicken flocks were further confirmed as *Salmonella* spp. by PCR amplification of 423bp of *InvA* gene. The lesions, cultural characteristics along with PCR amplification of *InvA* gene confirmed *Salmonella* spp. infection in desi chickens of Nagpur region.

***Keywords*:** Salmonellosis, desi chickens, pathology, molecular diagnosis

INTRODUCTION

Indian poultry industry is evolving and emerging as the world's second largest market; nevertheless, fowl Salmonellosis is becoming increasingly rampant and has a significant impact on the economy as well as the future development of poultry sector and backyard poultry farming. Avian salmonellosis is an important disease causing serious impediment to the development of poultry especially in developing countries of Asia[1]. The genus of *Salmonella* is a Gram-negative rod shaped bacteria belonging to the family of *Enterobacteriacea*. There are mainly two types of non-motile avian *Salmonella* spp. namely *Salmonella gallinarum* and *Salmonella pullorum* that cause fowl typhoid and pullorum disease, respectively. Besides, motile *Salmonellae* (paratyphoid group) infection causes Salmonellosis in chickens and has zoonotic significance[2].

Conventional bacterial culture methods are still used to identify *Salmonella* spp. and require about 3-11 days period. The standard culture methods for detecting *Salmonella* spp. in poultry include non-selective pre-enrichment followed by selective enrichment and plating on selective and differential agars. These methods are time consuming and labour intensive[3]. Polymerase chain reaction (PCR) assay have demonstrated their utility as screening tools for *Salmonella* testing in chickens[4]. *InvA* gene of *Salmonella* spp. has been proved as a suitable PCR target, with potential diagnostic applications[5]. Hence, present investigation was carried out to study the pathology and molecular detection of *Salmonella* spp. in desi chickens of Nagpur region which showed the symptoms and gross lesions suggestive of Salmonellosis.

MATERIALS AND METHODS

The study was conducted on total of six desi chicken farms having capacity of 500-1500

birds located in Nagpur region were mortality due to Salmonellosis occurred.

Necropsy examination

A total of 38 dead birds belonging to six desi farms suspected for Salmonellosis were subjected to detailed post mortem examination at the Department of Veterinary Pathology, Nagpur Veterinary Pathology, Nagpur and gross pathological lesions were recorded.

Histopathology

Tissue samples of liver, spleen and intestine were collected in 10% buffered formalin and processed for histopathological study by paraffin embedding technique. Sections were cut at 5-6 µ thickness and stained with routine haematoxylin and eosin (H and E) staining[6].

Isolation of bacteria

Loopful of samples from liver and intestinal contents were inoculated immediately in buffered peptone water (BPW) for pre enrichment and incubated at 37°C for 24 h. The samples were then inoculated into selective enrichment media Tetrathionate (TT) broth and incubated at 37°C for 18-24 h. After selective enrichment, one loopful of broth culture was streaked onto on to MacConkey lactose agar (MLA), brilliant green agar (BGA) and xylose-lysine-deoxycholate (XLD) agar and incubated at 37°C for 24 h[7].

Detection of *Salmonella* by PCR

Tissue samples of liver was also collected from the birds belonging to six desi chicken farms which showed gross lesions suspected of Salmonellosis and preserved at - 20°C. Bacterial DNA from tissue homogenate was extracted using HiGenoMB® genomic DNA Purification Kit (Himedia) as per the manufacturer's instructions. The *InvA* gene of *Salmonella* spp. from field samples were detected by using the forward (5'TCG TGA CTC GCG TAA ATG GCG ATA 3') and reverse primer (5'GCA GGC GCA CGC CAT AAT CAA TAA 3') to amplify *Salmonella* spp. specific 423 bp fragment[8]. For amplification, 3µl of DNA was incubated in total volume of 20 µl reaction mix containing 10 µl PCR master mix (2x), 1µl of each forward and reverse primer (10 pmol) and 5 µl of nuclease free water. PCR was carried out following initial denaturation at 95°C for 5 min and then 30 cycles at 94°C for 30 sec, 56°C for 1 min, and 72°C for 90 sec and a further extension at 72°C for 10 min.

RESULTS

Cultural characteristics of bacterial isolates

Cultural examination showed colorless, translucent, smooth and raised colonies on MLA indicative of lactose non-fermenter organisms. On BGA, the Salmonella isolates showed pinkish white colonies with change of the colour of agar medium from green to pink. While on XLD agar, red colonies were produced initially after 24 h of incubation, which get blackened at center on prolonged incubation (Fig. 1). On the basis of the cultural and morphological characteristics, *Salmonella* spp. infection was confirmed in six desi chicken flocks.

Gross pathology

Chicks affected with Salmonellosis showed unabsorbed, coagulated and greenish discolouration of yolk. Liver showed congestion and rounded borders along with hepatomegaly. In most of the cases, whitish necrotic foci were observed on entire parenchyma of liver while in few cases, numerous haemorrhagic foci were noticed (Fig.2). Spleen revealed congestion with or without splenomegaly. Splenomegaly along with mottling was also evident during necropsy. Small intestine showed catarrhal enteritis (Fig. 3), while typhilitis without caecal core were also noticed in caeca.

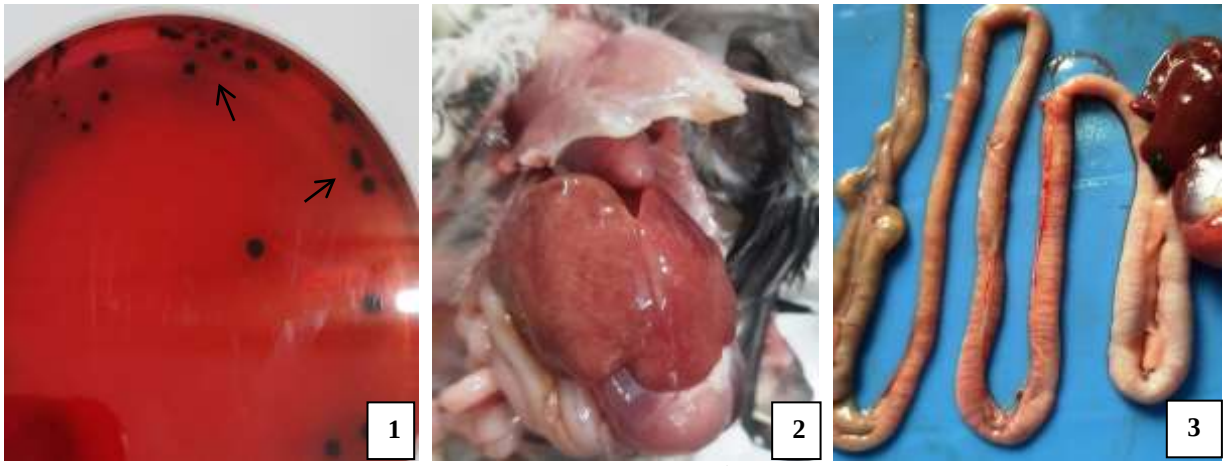

Fig.1 Jet black colour colonies on XLD agar. 2. Liver showing whitish necrotic foci on entire parenchyma. 3. Intestine showing catarrhal enteritis.

Histopathology

Liver showed congestion, hemorrhages and dilatation of sinusoids, vacuolar degeneration in hepatocytes and leucocytic infiltration (Fig. 4). Spleen showed depletion of lymphocytes in follicle of white pulp with reticuloendothelial cell hyperplasia. Intestine showed haemorrhages, goblet cell hyperplasia and sloughing or desquamation of vili epithelium (Fig. 5). Intense heterophilic and leucocytic infiltration in the submucosa of small intestine and caeca was observed.

Molecular detection by PCR

Amplification of *InvA* gene of *Salmonella* spp. revealed 423bp product for all six desi chicken flocks (Fig. 6). Routine PCR test in conjunction with traditional identification methods could be effective in providing a more accurate profile for prevalence of *Salmonella* and detection of Salmonella spp. in poultry flocks. Poor samples quality and delayed transport media make the cultural diagnosis difficult and tedious. Hence, nucleic acid based techniques are considered as the best alternative tools for easy and rapid confirmatory diagnosis of Salmonellosis.

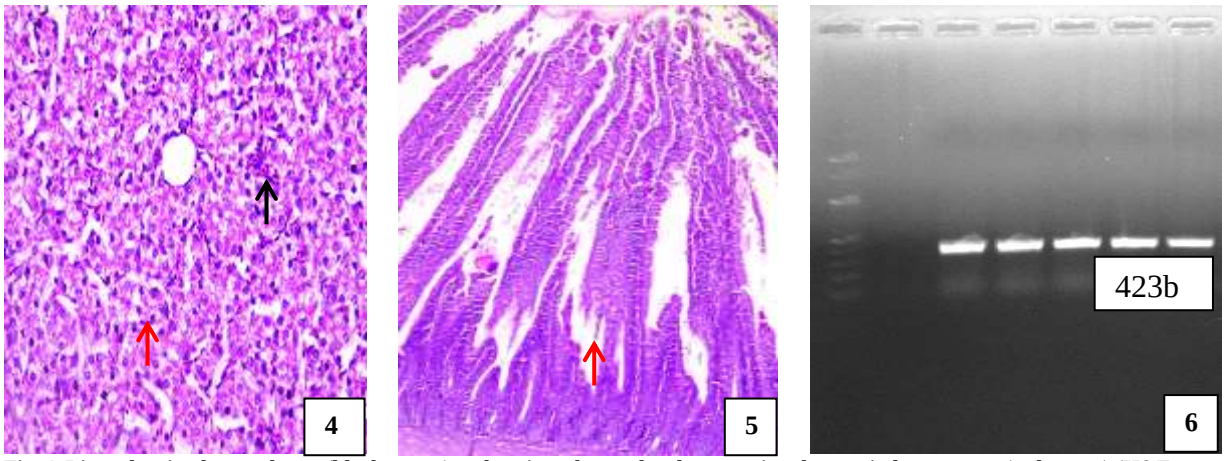

Fig. 4. Liver showing hemorrhages (black arrow), pyknosis and vacuolar degenerative changes in hepatocytes (red arrow) (H&E, 20x). 5. Duodenum showing desquamation of villi (red arrow) (H&E, 20x). 6. Agarose gel photograph showing amplified PCR products of *Salmonella* spp. Lane 1: 100bp DNA ladder, Lane 2: Negative control, Lane 3 to 7: positive field sample (423bp of *InvA* gene)

DISCUSSION

Characteristic colorless, translucent, smooth and raised colonies on MLA, pinkish white colonies on BGA and jet black colonies on XLD agar is indicative of *Salmonella* spp. infection in all six desi chicken flocks. The

cultural morphology observed on MLA, BGA and XLD is in accordance with the previous researchers [9, 10].

Gross lesions noticed in various visceral organs are in agreement with the previous researchers. Focal hemorrhages in liver of birds with Salmonellosis were also reported by researcher[11]. Necrotic foci and hepatomegaly in cases of *Salmonella* spp. infection was reported by previous researcher[12]. Furthers, lesions observed in liver during present study were also in agreement with previous obervations[13,2]. Enlarged and congested spleen in birds with Salmonellosis was also reported by researchers[11, 14]. Lesion of catarrhal enteritis and typhilitis noticed during present investigation are in accordance with researcher[15,16].

Histopathological lesions noticed in liver are in agreement with worker[17] who also noticed similar lesions during *Salmonella* spp. infection in chickens. Depletion of lymphocytes in white pulp with reticuloendothelial cell hyperplasia observed in present study was also reported previous researcher [18]. Microscopic lesions observed in intestine were comparable with researcher[19.]

Amplification of 423bp of *InvA* gene by PCR confirmed the *Salmonella* spp. infection in six desi chicken flocks. These findings are in accordance with previous researcher[5] who also detected *InvA* gene of *Salmonella* spp. in birds. Further, it is also reported that *InvA* gene was able to identify *Salmonella* spp. by PCR assay[20]. The *invA* is only found in *Salmonella* spp. and is regarded as a golden marker in detection and genetic characterization of *Salmonella* spp[21].

CONCLUSION

Gross and histopathological lesions, cultural characteristics along with PCR amplification of *InvA* gene suggested the outbreak of *Salmonella* spp. in desi chickens farms. PCR based technique is considered as the best alternative tool for easy and rapid confirmatory diagnosis of *Salmonella* spp. infection in chickens.

Declaration by Authors

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