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Original Research

# Pathology and Molecular Detection of Salmonellosis in Commercial Chickens of Chhattisgarh

**P. M. Sonkusale[1]*, R.C. Ghosh[1], Devesh Kumar Giri[1], Vivek Kumar[1], Poornima Gumasta[1], Neha Shukla[1] and Savita Bisen[2]**

[1]Department of Pathology, College of Veterinary Science & A. H. Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg, Chhattisgarh INDIA
[2]Department of Parasitology, College of Veterinary Science & A. H. Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg, Chhattisgarh INDIA

*Corresponding Author: drprashantvet@yahoo.com

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## Abstract

*Avian salmonellosis is an important disease-causing serious impediment to the development of the poultry industry especially in developing countries. During the present study, mortality of 16 to 22% due to Salmonellosis in thirteen, 1-2 weeks old commercial chicken flocks located at Durg, Rajnandgaon, and Raipur district of Chhattisgarh was noticed. Characteristic clinical signs of depression, anorexia, huddling, droopy wings, dehydration, laboured breathing, diarrhoea, and adherence of faeces to the vent were suggestive of Salmonellosis. Necrotic foci on the liver, omphalitis, and catarrhal enteritis were the characteristic lesions found in Salmonella-affected birds. Colorless colonies on MLA, light pink colonies on BGA, and jet-black colonies on XLD agar confirmed the Salmonella infection. Clinical samples of liver collected from thirteen commercial chicken flocks were further confirmed as Salmonella spp. by PCR amplification of 423bp of InvA gene. The clinical signs, lesions, and cultural characteristics along with PCR amplification of InvA gene confirmed Salmonella infection in commercial chickens of Chhattisgarh.*

**Keywords:** Commercial Chickens, Pathology, Molecular Diagnosis, Salmonellosis.

## Introduction

Poultry farming in India has a vital role to play in the development of socio-economic conditions of the people by providing employment opportunities, more earning and human food of high nutritive value. India is one of the top countries in the world in the production and export of poultry produce. Infections are of great importance for commercial chickens, as they cause heavy economic losses by way of high mortality and decreased egg production, and reduced fertility and hatchability.

Avian salmonellosis is an important disease-causing serious impediment to the development of the poultry industry especially in developing countries of Asia (Rajagopal and Mini, 2013). The genus of *Salmonella* is a Gram-negative rod-shaped bacterium belonging to the family of *Enterobacteriacea*. There are mainly two types of non-motile avian *Salmonella* spp. namely *Salmonella gallinarum* and *Salmonella pullorum* which cause fowl typhoid and pullorum disease, respectively. Besides, motile *Salmonellae* (paratyphoid group) infection causes Salmonellosis in chickens and has zoonotic significance (Hossain *et al.,* 2006). 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 (Menghistu *et al*., 2011). Polymerase chain reaction (PCR) assays have demonstrated their utility as screening tools for *Salmonella* testing in commercial chickens (Bautista et al., 2011). The *InvA* gene of *Salmonella* has been proven as a suitable PCR target, with potential diagnostic applications (Shanmugasamy *et al.,* 2011). Hence, the present investigation was undertaken to confirm Salmonellosis by PCR technique in commercial chickens of Chhattisgarh which showed the symptoms and gross lesions suggestive of Salmonellosis.

## Materials and Methods

Mortality due to Salmonellosis in 1-2 weeks old commercial chicken farms with a capacity of 2000-10000 birds were observed in Durg and Rajnandgaon and Raipur districts of Chhattisgarh. A total of thirteen commercial farms where mortality was suspected due to Salmonellosis was visited and information about age, flock size, and mortality was collected.

**Clinical Signs and Gross Pathology:** The ailing birds were examined for clinical signs, if any. Dead birds were subjected to detailed post-mortem examination and gross pathological lesions were recorded.

**Histopathology:** Tissue samples of the 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 (Luna, 1968).

**Bacterial Isolation:** Loopful of samples from the liver were inoculated immediately in Rappaport Vassiliadis Soya (RVS) peptone broth for selective enrichment of Salmonella organisms and incubated at 37°C for 24 h. After selective enrichment, one loopful of each RVS culture was streaked onto MacConkey lactose agar (MLA), brilliant green agar (BGA), and xylose-lysine-deoxycholate (XLD) agar and incubated at 37°C for 24 h (OIE, 2012). The non-lactose fermenting colonies of MLA were characterized microscopically using Gram's-stain.

**Detection of *Salmonella* by PCR:** Tissue samples of the liver were also collected from the birds belonging to thirteen commercial chicken farms which showed gross lesions suspected of Salmonellosis and preserved at - 20ºC for detection of *InvA* gene of *Salmonella* spp. by **PCR.** 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 was detected by using the forward primer 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 (Nair *et al.,* 2015). For amplification, 3µl of DNA was incubated in the 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. The PCR products were separated in 1.5% agarose gel and visualized in Geldoc (Biorad).

## Results and Discussion

Mortality of about 16-22% due to Salmonellosis in 1 to 2 weeks old commercial chickens were recorded at thirteen farms located at Durg, Rajnandgaon and Raipur districts of Chhattisgarh. Affected birds showed signs of depression, somnolence, anorexia, huddling, droopy wings, laboured breathing, diarrhoea, dehydration, ruffled feathers, weakness, and adherence of faeces to the vent. These clinical signs are in accordance with Shivaprasad, (2000) and Soufy *et al.*, (2016).

**Cultural And Staining Characteristics of Bacterial Isolates:** Gram-staining revealed the presence of small, rod shape Gram-negative bacteria arranged singly and in pair. On MLA, colorless, translucent, smooth, and raised colonies indicative of lactose non-fermenter organisms were observed. On BGA, non-lactose fermenter isolates produced a light pink colony against a rose-pink background. While on XLD agar, red colonies were produced initially after 24 h of incubation, which get blackened at the center on prolonged incubation (Fig. 3). Cultural morphology on XLD, MLA and BGA was in accordance with the findings of Ferdous *et al.*, (2013) and Sannat *et al.*, (2017).

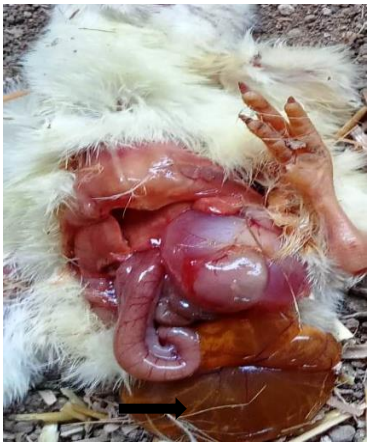

**Fig.1**Unabsorbed, coagulated and greenish discoloration of yolk in broiler chick

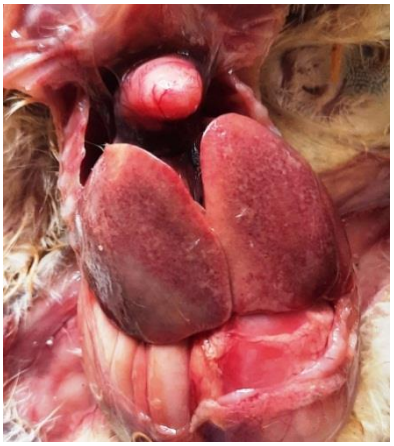

**Fig. 2** Liver showing necrotic foci in chick

**Fig. 3** Jet black colour colonies on XLD agar

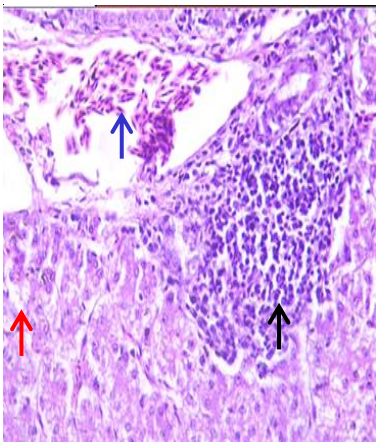

**Fig. 4** Liver showing congestion (blue arrow), leucocytic infiltration (black arrow) and vacuolar degenerative changes in hepatocytes (red arrow) (H&E, 20x**)**

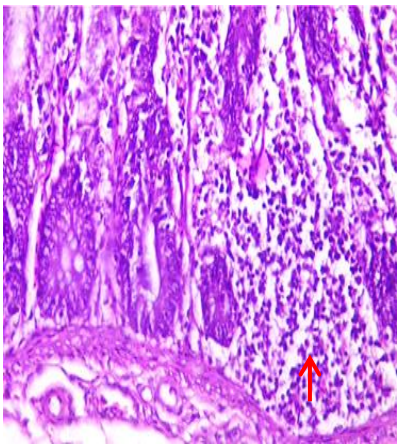

**Fig. 5** Ileum showing leucocytic infiltration in submucosa (red arrow) (H&E, 20x)

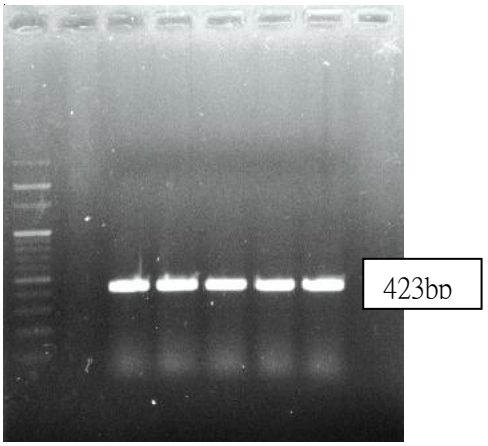


**Fig. 6** Agarose gel photograph showing amplified PCR products of *Salmonella* spp. Lane 1: 100bp DNA ladder, Lane 2: Negative control, Lane 3,4,5,6,7: positive field sample (423bp of *InvA* gene)

**Gross Pathology:** Birds affected with Salmonellosis showed unabsorbed, coagulated and greenish discoloration of yolk (Fig.1). Liver showed congestion and rounded borders along with hepatomegaly. In most of the cases, whitish necrotic foci were observed on the entire parenchyma of the liver while in a few cases, numerous haemorrhagic foci were noticed (Fig.2). Focal haemorrhages in the liver of birds with Salmonellosis were also reported by Nazir *et al*., (2012). Necrotic foci and hepatomegaly in cases of *Salmonella* infection were reported by Ahmed *et al.,* (2008). Further, lesions observed in the liver during the present study were also in agreement with Bhattacharya *et al.*, (2001) and Hossain *et al*., (2006). Spleen revealed congestion with or without splenomegaly. Splenomegaly along with mottling was also evident during necropsy. Similar findings of enlarged and congested spleen in birds with Salmonellosis were reported by Nazir *et al.,* (2012) and Kumari *et al*., (2013). The small intestine showed catarrhal enteritis while typhilitis without caecal core were noticed in the caeca. These findings are in accordance with Hooda *et al*., 2011 and Alhenaky *et al.,* 2017.

Histopathology: The liver showed haemorrhagic foci, congestion and dilatation of sinusoids, vacuolar degeneration in hepatocytes, and leucocytic infiltration (Fig. 4). These findings are in agreement with Ogunleye *et al.,* (2012) who noticed similar lesions during *Salmonella* infection in chickens. Spleen showed a depletion of lymphocytes in the follicle of white pulp with reticuloendothelial cell hyperplasia. Similar findings were reported by Saha *et al.*, (2012). The intestine showed haemorrhages, goblet cell hyperplasia, and sloughing or desquamation of villi epithelium. Intense heterophilic and lymphocytic infiltration in the submucosa of the small intestine (Fig. 5) and caeca was observed. These findings were comparable with the microscopic lesions observed by Islam *et al.,* (2006).

**Polymerase Chain Reaction:** Tissue samples of liver collected from thirteen commercial flocks were confirmed as *Salmonella* spp. infection by PCR. Amplification of *InvA* gene of *Salmonella* spp. revealed 423bp product for all thirteen commercial flocks (Fig. 6). These findings are in accordance with Shanmugasamy *et al.,* (2011) who also detected *InvA* gene of *Salmonella* spp. in birds**.** Further, Oliveira *et al.,* (2003) and Abd El-Ghany *et al.,* (2012) reported that *InvA* gene was able to identify *Salmonella* spp. and routine PCR tests in conjunction with traditional identification methods could be effective in providing a more accurate profile for the prevalence of *Salmonella* in poultry flocks. Poor sample 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 (Shanmugasamy *et al.*, 2011).

## Conclusions

The clinical signs, gross and histopathological findings, and cultural characteristics along with PCR amplification of *InvA* gene suggested the outbreak of Salmonellosis in commercial chicken farms of Chhattisgarh. In fact, necrotic foci on the liver, omphalitis, and catarrhal enteritis were the important gross lesions observed in *Salmonella-affected* birds. *InvA* gene-based PCR for detection of *Salmonella* spp. is the simplest and less expensive method and is advantageous when compared with conventional cultural methods.

## Contribution by Authors

Equal contribution

## Conflict of Interests

There is no conflict of interest.

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## References

1. Abd El-Ghany, W. A., El-Shafii,S.S.A. and Hatem, M. E. (2012) A survey on *Salmonella* species isolated from chicken flocks in Egypt. *Asian Journal of Animal and Veterinary Advances*, 7(6): 489-501.
2. Ahmed, A.K.M., Islam, M.T., Haider, M.G. and Hossain, M. M. (2008) Sero-prevalence and pathology of naturally infected Salmonellosis in poultry with isolation and identification of causal agents. *Journal of*

*Bangladesh Agricultural University,* 6: 327-334.
3. Alhenaky, A., Abdelqader, A., Abuajamieh, M. and Al-Fata, A.R. (2017) The effect of heat stress on intestinal integrity and *Salmonella* invasion in broiler birds. *Journal of Thermal Biology,* 70: 9-14.
4. Bautista, D.A., Sample, B., Phillips, K., Wright, B.J. and Sullivan, L. (2011) Performance evaluation of *Salmonella* PCR screening assays in poultry samples. *Proceeding of 16th Western Poultry Disease Conference*, pp: 20- 24.
5. Bhattacharya, A., Majumder, P. and Dutta, M. K. (2001) Isolation, characterization and antibiotic spectra of *S. gallinarum* from an outbreak of fowl typhoid in adult broiler parent flock in Tripura. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases,* 22: 56-58.
6. Ferdous, T. A., Lutful, K.S.M., Mansurul A. M. and Mahmud H. K.M. (2013) Identification and antimicrobial susceptibility of *Salmonella* species isolated from washing and rinsed water of broilers in pluck shops. *International Journal of Animal and Veterinary Advances,* 5(1): 1-8.
7. Hooda, A., Mishra, S.K., Nehra, V. and Lather, D. (2011) Pathoanatomical studies on poultry mortality with special reference to gastro intestinal disorders. *Haryana Veterinary,* 50: 80-84.
8. Hossain, M. S., Chowdhury, E. H., Islam, M. M., Haider, M. G. and Hossain, M. M. (2006) Avian Salmonella infection: isolation and identification of organisms and histopathological study. *Bangladesh Journal of Veterinary Medicine,* 4 (1): 07–12.
9. Islam, M.M., Haider, M.G., Chowdhury, E.H., Kamruzzaman, M. and Hossain, M.M. (2006) Seroprevalence and pathological study of *Salmonella* infections in layer chickens and isolation and identifi cation of causal agents. *Bangladesh Journal of Veterinary Medicine,* 4: 79-85.
10. Kumari, D., Mishra, S.K. and Lather, D. (2013) Pathomicrobial studies on *Salmonella gallinarum* infection in broiler chickens. *Veterinary World,* 6: 725-729.
11. Luna, A. G. (1968) Manual of histology and special staining technique. 3rd Ed, 1, Armed Force Institute of Pathology-Magraw Hill Book Co, London.
12. Menghistu ,H.T., Rathore, R., Dhama, K. and Agarwal, R.K.(2011) Isolation, Identification and Polymerase chain reaction (PCR) detection of *Salmonella* species from field materials of poultry origin. *International Journal of Microbiological Research,* 2 (2): 135-142.
13. Nair, A., Balasaravanan, T., Malik, S.V.S., Mohan, V., Manesh, K., Vergis, J. and Rawool, D.B. (2015) Isolation and identification of *Salmonella* from diarrheagenic infants and young animals, sewage waste and fresh vegetables. *Veterinary World,* 8(5):669-73.
14. Nazir, S., Kamil, S.A., Darzi, M.M., Mir, M.S. and Amare, A. (2012) Pathology of spontaneously occurring Salmonellosis in commercial broiler chickens of Kashmir valley. *Journal of World Poultry Research,* 2: 63-69.
15. Ogunleye, A.O. and Carlson, S.A. (2012) Emergence of an SGI1-bearing *Salmonella* enterica serotype Kentucky isolated from septic poultry in Nigeria. *The Journal of Infection in Developing Country,* 6: 483-488.
16. OIE. Fowl typhoid and Pullorum disease Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 7th ed. Paris: *Office International Des Epizooties*, 2012. pp. 1–14.
17. Oliveira, S.D., Rodenbusch, C.R., Ce, M. C., Rocha, S.L.S. and Canal, C.W. (2003) Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. *Letters in Applied Microbiology,* 36: 217–221.
18. Rajagopal, R. and Mini, M. (2013) Outbreaks of Salmonellosis in three different poultry farms of Kerala, India. *Asian Pacific Journal of Tropical Biomedicine,* 3(6): 496-500.
19. Saha, A.K., Sufian, M.A., Hossain, M.I. and Hossain, M.M. (2012) Salmonellosis in layer chickens: pathological features and isolation of bacteria from ovaries and inner content of laid eggs. *Journal of Bangladesh Agricultural University*, 10: 61-67.
20. Sannat, C., Patyal, A., Rawat, N., Ghosh, R.C., Jolhe, D.K., Shende, R.K., Hirpurkar, S.D and Shakya, S. (2017) Characterization of *Salmonella Gallinarum* from an outbreak in Raigarh, Chhattisgarh. *Veterinary World*, 10(2): 144-148.
21. Shanmugasamy, M., Velayutham, T. and Rajeswar, J. (2011) *Inv A* gene specific PCR for detection of *Salmonella* from broilers. *Veterinary World*, 4(12):562-564.
22. Shivaprasad, H. L. (2000) Fowl typhoid and pullorum disease. *Revue scientifique et technique (International Office of Epizootics),* 19: 405-424.
23. Soufy, H., Gab-Allah, M.S., Tantawy, A. A., Fotouh, A. and Soad, M. N. (2016) Pathogenesis of Experimental *Salmonella gallinarum* Infection (fowl typhoid) in Broiler Chicks. *Egyptian Journal of Veterinary Science*, 47: (2) 117 – 131.

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