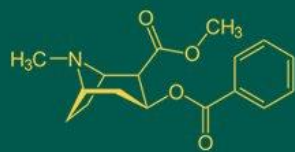


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(6): 1097-1104
www.biochemjournal.com
Received: 02-03-2026
Accepted: 06-04-2026

Pradeep Dubey
MVSc., Department of
Veterinary Pathology, College
of Veterinary Science and
Animal Husbandry, Dau Shri
Vasudev Chandrakar
Kamdhenu Vishwavidyalaya,
Durg, Chhattisgarh, India

Dhananjay Kumar Jolhe
Associate Professor & Head,
Department of Veterinary
Pathology, College of
Veterinary Science and Animal
Husbandry, Dau Shri Vasudev
Chandrakar Kamdhenu
Vishwavidyalaya, Durg,
Chhattisgarh, India

Ratan Chandra Ghosh
Professor, Department of
Veterinary Pathology, College
of Veterinary Science and
Animal Husbandry, Dau Shri
Vasudev Chandrakar
Kamdhenu Vishwavidyalaya,
Durg, Chhattisgarh, India

Prashant M. Sonkusale
Associate Professor & Head,
Department of Veterinary
Pathology, Nagpur Veterinary
College, Nagpur, Maharashtra,
India

Vivek Kumar
PhD Scholar, Division of
Pathology, ICAR-Indian
Veterinary Research Institute
Bareilly, Uttar Pradesh, India

Corresponding Author:
Vivek Kumar
PhD Scholar, Division of
Pathology, ICAR-Indian
Veterinary Research Institute
Bareilly, Uttar Pradesh, India

Pathology, diagnosis, and molecular characterization of salmonellosis in Kadaknath chickens

Pradeep Dubey, Dhananjay Kumar Jolhe, Ratan Chandra Ghosh, Prashant M Sonkusale and Vivek Kumar

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6n.8833>

Abstract

The investigation was aimed to assess the prevalence, pathology, and molecular diagnosis in Kadaknath chickens. Data collected over 6 months, from December 2020 to June 2021, in Durg, Rajnandgaon, Raipur, and Balod, Chhattisgarh. Kadaknath birds exhibited signs such as anemia, depression, labored breathing, and diarrhea. The highest pullorum disease mortality occurs in 2–3-week-old birds; older birds may show mild symptoms. Breeding and laying flocks show increased susceptibility at the lay point, with reduced egg production as the only sign. Out of 26 cloacal swabs from Durg, Rajnandgaon, Balod, and Raipur, 6 were found positive by PCR. Blood samples from Kadaknath birds showed no significant changes. Suspected salmonellosis deaths showed hepatomegaly, congestion, hemorrhages, and necrotic foci. Spleens showed splenomegaly with necrotic foci. Intestines and caeca had dilation and yellowish exudate. Liver histopathology showed degeneration, necrosis, congestion, hemorrhages, and inflammation. Spleen sections depicted degeneration, necrosis, and lymphocyte depletion. Intestinal sections showed villi denudation, necrosis, and infiltration. Genes *I137_08605* and *ratA ROD* were targeted for multiplex PCR to identify 8 field isolates. Only 3 isolates produced a 290 bp amplicon, confirming *S. gallinarum* and/or *S. pullorum*, with 3 also confirming *S. gallinarum* via a 571 bp amplicon. Durg's *Salmonella* Gallinarum showed 99.82% similarity with Brazil's *Salmonella* enterica subsp. enterica serovar Abaetetuba strain. Brazil's strain (Acc. No. ATCC 35640) showed 99.82% identity with *Salmonella* Gallinarum Durg. *Salmonella* Durg *ROD* *ratA* gene protein sequence alignment showed 99.82% identity with *Salmonella* Brazil. Phylogenetic analysis indicated that *Salmonella* Durg and Brazil share a common ancestor. In silico protein translation of the *ratA ROD* nucleotide sequence from Durg's *Salmonella* Gallinarum revealed a 190 amino acid sequence.

Keywords: Gallinarum, Histopathology, PCR, Pullorum, *ratA ROD*, *Salmonella*

Introduction

Salmonella is a major zoonotic pathogen affecting both animals and humans (Li Q Wang *et al.*, 2020) [33]. Infections in poultry reduce production and contaminate the food chain, leading to economic losses and public health risks (Syedjmani D *et al.*, 2016). Despite control measures like vaccination (Zhang-Barber L *et al.*, 1999) [37], *Salmonella* remains a global challenge, as survivors become permanent carriers (Shivprasad and Barrow, 2008). Over 2,600 serotypes have been identified (Hendriksen *et al.*, 2011) [13], exhibiting similar genomes but different host ranges. Specifically, *S. gallinarum* and *S. pullorum* cause fowl typhoid and pullorum disease, resulting in significant losses in the poultry industry, particularly in China (Soria *et al.*, 2012) [30]. Meanwhile, *S. Typhimurium* and *S. Enteritidis* are prevalent food-borne pathogens; *S. Typhimurium* causes typhoid-like illness, while *S. Enteritidis* is a leading cause of human gastroenteritis (Vugia DJ *et al.*, 1999). Other serotypes, such as *S. Kentucky* and *S. Newport*, are also commonly found in poultry (Long *et al.*, 2016) [18]. Fowl typhoid and Pullorum disease are globally prevalent, particularly in developing nations where antimicrobial resistance is increasing. Free-range systems may elevate infection risks. In India, Salmonellosis is documented across multiple regions. Transmission occurs *trans*-ovarially or horizontally via hatcheries and contaminated litter, with wild birds acting as reservoirs. *Salmonella* causes bacteraemia and septicaemia through cellular invasion and multiplication. Clinical signs in young chicks include high mortality and poor quality, while older birds exhibit anaemia, respiratory distress, and diarrhoea.

Breeding flocks often experience reduced egg production and hatchability. Pathological lesions range from peritonitis and inflamed yolk sacs in chicks to misshapen ovaries in adults, alongside septicaemic indicators like liver enlargement. Diagnosis is based on flock history, clinical presentation, and laboratory culture confirmed by biochemical testing. PCR technology represents an exceptionally rapid and highly sensitive alternative for clinical diagnosis, offering the robust capability to detect and differentiate multiple serovars simultaneously from a single biological sample (Xiong *et al.*, 2018) [35]. To date, there is a significant lack of comprehensive benchmark data or systematic epidemiological studies regarding the prevalence of Pullorum disease and Fowl typhoid specifically within the Kadaknath chicken populations found in the state of Chhattisgarh. As per recent studies, the prevalence of Salmonellosis in Kadaknath farms located in the Durg, Raipur, and Rajnandgaon districts has been observed to be at rates of

9.2%, 7.9%, and 5.2%, respectively (Pradeep *et al.*, 2026) [22]. At present, diagnostic efforts for Salmonellosis in this region rely almost exclusively on traditional, time-consuming phenotypic methods which often lack the necessary precision. Consequently, there is an urgent and clear necessity to develop and implement a standardized molecular diagnosis protocol to accurately identify Pullorum disease and Fowl typhoid. This study focuses on the detailed molecular characterization of *Salmonella pullorum* and *Salmonella gallinarum* species to enhance diagnostic accuracy and disease management.

The study was conducted with following objectives:

- To examine pathomorphological changes in chicken organs due to Salmonellosis.
- Molecular characterization of *Salmonella pullorum* and *Salmonella gallinarum* species.

Materials & Methods

Table 1: Details of primers used in PCR for *S. pullorum* and *S. gallinarum*

Gene	Primers	Size	Salmonella Sp.
I137_08605	5' – CACTGGAGACTCTGAGGACA-3' F	290 bp	Detects both <i>S. pullorum</i> and <i>S. gallinarum</i>
I137_08605	5' –GGGCAGGGAGTCTTGAGATT-3' R		
ratA	5'-ATTGCTCTCGTCCTGGGTAC-3' F	571bp	Detects only <i>S.gallinarum</i>
ratA	5'-TACCGATACGCCCAACTACC-3' R		

Table 2: PCR reaction mixture for *S. pullorum* and *S. gallinarum*

S.No.	Components	Volume
1	2X PCR TaqMixture	5 µL
2	Forward Primer (10 pmol)	0.5 µL
3	Reverse Primer (10 pmol)	0.5 µL
4	DNA template	2 µL
5	Nuclease free water	2 µL
	Total volume	10 µL

Table 3: PCR protocol for *S. pullorum* and *S. gallinarum*

S. No.	Steps	Temperature and time	Cycles
1	Initial denaturation	94°C for 5 minutes	
2	Denaturation	94°C for 45 second	30 cycles
3	Annealing	52°C for 45 second	
4	Extension	72°C for 40 second	
5	Final extension	72°C for 10 minutes	

Amplification of *I137_08605* and *ratA* genes included initial denaturation at 94 °C for 5 min, 30 cycles of 94 °C for 45 s, 52 °C for 45 s, and 72 °C for 40 s, with a final extension at 72 °C for 10 min (Table.3). PCR amplicons were analyzed by agarose gel electrophoresis (BIORAD, UK).

Protocol

Agarose (GeNei, Bangalore) was boiled with 1X TAE buffer (GenEi, Bangalore) to prepare 2% agarose gel. After cooling to 50 °C, ethidium bromide (fc .5µg/ml) was added. The gel was poured into a casting tray, allowed to solidify, and submerged in 1X TAE buffer. A comb was placed to form sample slots, then carefully removed after solidification. PCR products were mixed with 6X loading dye (HIMEDIA, Mumbai) and loaded into wells. A 100 bp

DNA ladder was used as a marker (Bioserve Bio Technologies Pvt. Ltd. Hyderabad, India). Electrophoresis was at 5 volts/cm; DNA migration was observed by UV transilluminator and documented (GEL DOC – EZ BIORAD, UK).

Sequencing of rat A ROD gene

PCR products of positive *Salmonella enterica* subsp. *Enterica serovar gallinarum* samples were sent to Arraygen for purification and sequencing (Forward and Reverse strands). The sequence data was received as electropherograms and Fasta text files. Sequenced files were loaded into MEGA 7 software. The reverse complement of the reverse strand was generated from

https://www.bioinformatics.org/sms/rev_comp.html.

Nucleotide sequences were aligned using NCBI multiple alignment tool and compared with *Salmonella enterica* sequences. The consensus sequence of rat A ROD gene was derived. Rat A ROD gene analysis was done using an online tool. The sequence was uploaded to the ExPASy server (www.expasy.org/tool) for primary structure analysis.

Phylogenetic analysis

The consensus sequence of rat A ROD gene of *S. gallinarum* was used for homology search with BLAST. Multiple sequence alignment was done using Clustal W, and a phylogenetic tree was generated using MEGA X (Kumar *et al.*, 2018) [15] neighbor method (Saitou and Nei, 1987) [23]. All sequences were retrieved from GENBANK for analysis. Accession number from GENBANK was Banklt2529837 BSeq#1 OL871236.

Table 4: Cloacal Swab Analysis of Kadaknath birds

S No	Districts	No of Samples collected from birds showing signs of Salmonellosis	Overall positive samples based on PCR assay
1.	Durg	10	03
2.	Rajnandgaon	06	02
3.	Balod	05	00
4.	Raipur	05	01
Total		26	06

Out of 26 Cloacal swab samples from Durg, Rajnandgaon, Balod and Raipur districts examined, 06 samples were found positive on the basis of PCR assay.

Clinical signs

Total of 210 dead birds showed signs like decreased feed intake, depression, huddling, droopy wings, labored breathing, diarrhea, dehydration, and ruffled feathers, suspected for Salmonellosis. These findings matched Shivaprasad (2000) [28], who reported similar signs in fowl typhoid and pullorum disease, including depression, somnolence, anorexia, huddling, droopy wings, dehydration, labored breathing, diarrhea, ruffled feathers, weakness, and soiled vent. Barrow *et al.* (2012) [1], Soufy *et al.* (2016) [29],

Blackall *et al.* (2008) [2], and Haider *et al.* (2014) [12] also reported similar signs in Salmonellosis-affected chickens.

Gross pathology

Most birds with Salmonellosis showed hepatomegaly, congestion, hemorrhages, and necrotic foci (Fig. 2), consistent with findings by Freitas Neto *et al.* (2007) [9], Haider *et al.* (2008), Berchieri and Freitas (2009), and Garcia *et al.* (2013) [10]. Characteristic lesions included a friable liver with bronze discoloration and necrotic foci, similar to those reported by Saha *et al.* (2012) [24]. The spleen was enlarged with congestion and necrotic foci (Fig. 3), aligning with Haider *et al.* (2008) and Garcia *et al.* (2013) [10]. Intestinal examination revealed congestion, hemorrhages, and enteritis (Fig. 4). The intestines and caeca showed dilation with yellowish exudates (Fig. 4), and chronic cases exhibited typhlitis with necrotic casts, agreeing with several studies (Hafeji *et al.*, 2001; Syed *et al.*, 2004; Wigley *et al.*, 2005; Hossain *et al.*, 2006; Islam *et al.*, 2006; Saha *et al.*, 2012; Fotouh *et al.*, 2014) [11, 31, 34, 14, 16, 24, 8]. Since these clinical and post-mortem signs are not unique to specific *Salmonella* infections, definitive diagnosis requires organism isolation, serological testing, and PCR assay (OIE, 2012) [21].



Fig 1: Photograph of Salmonellosis affected bird showing congestion with multiple white necrotic foci



Fig 2: Photograph of liver of Salmonellosis affected bird showing congestion and hepatomegaly



Fig 3: Photograph of spleen of Salmonellosis affected bird showing splenomegaly with white necrotic foci



Fig 4: Photograph of caeca of Salmonellosis affected bird showing dilatation with yellowish catarrhal exudate.

Histopathological examination

Histopathological examination of liver sections from birds affected by Salmonellosis revealed extensive degenerative alterations, including vacuolar degeneration and coagulative necrosis, accompanied by vascular congestion, hemorrhages, and inflammatory cell infiltration. Notable hepatocellular necrosis was characterized by eosinophilic hepatocytes and pyknotic nuclei. These pathological findings are consistent with the lesions reported by Soufy *et al.* (2016) [29] in broiler chicks challenged with *S. gallinarum*, as well as observations documented by Hafeji *et*

al. (2001)^[11], Hossain *et al.* (2006)^[14], Wigley *et al.* (2005)^[34], and Casagrande *et al.* (2014)^[5]. Similar manifestations were also recorded in chickens infected with *S. gallinarum* by Prasanna and Paliwl (2003). The virulence of *S. gallinarum* has been attributed to the presence of a virulence plasmid (Bailey and Scott, 1990). The pathogenesis likely involves initial intestinal localization following ingestion, subsequently progressing to bacteremia and hepatitis. Spleen sections exhibited degeneration, necrosis, and lymphocyte depletion within germinal centers, with certain cases demonstrating reticuloendothelial cell proliferation. These splenic lesions align with the findings of Haider *et al.* (2008), who reported congestion and lymphocytic necrosis in pullets infected with *Salmonella enterica* subspecies Pullorum. Intestinal sections displayed denudation of villi, necrosis, mononuclear cell (MNC) infiltration, and villous sloughing (Fig 5). These intestinal and cecal observations are in accordance with results published by Syed *et al.* (2004)^[31], Wigley *et al.* (2005)^[34], Hossain *et al.* (2006)^[14], Saha *et al.* (2012)^[24], and Fotouh *et al.* (2014)^[8]. Many organisms exhibit resistance to intestinal acidity and proteolytic enzymes, being subsequently shed through feces. *Salmonella* organisms adhere to mannose-like receptors on the microvilli of epithelial cells. Localized microvilli degeneration at the attachment sites facilitates bacterial entry, thereby breaching the cell surface. Following epithelial penetration, the pathogenicity is contingent upon bacterial multiplication, systemic spread, toxin production, cellular damage, and inflammatory responses.

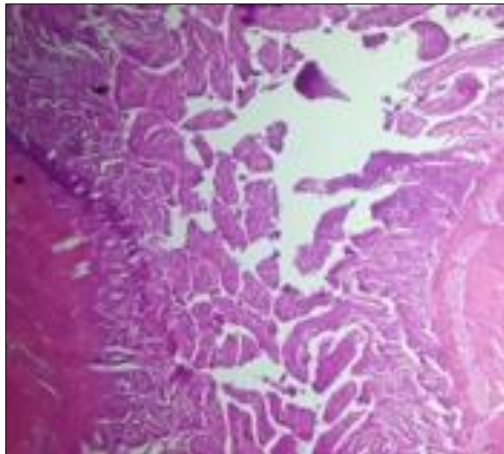


Fig 5: Photomicrograph of Intenstine showing Sloughing and denudation of villi. (H & E X100)

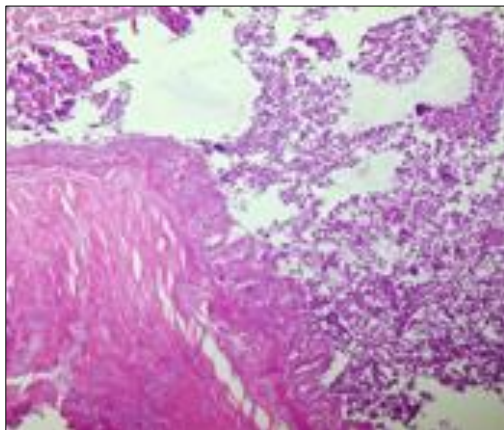


Fig 6: Denudation and clubbing of intestinal villi with necrotic debris and infiltration of mononuclear cells in the lumen

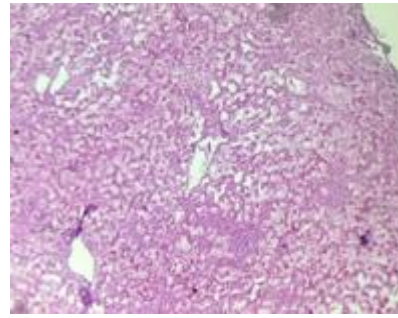


Fig 7: Dialatation of sinusoidal spaces and vacuolation of hepatocytes with necrotic changes

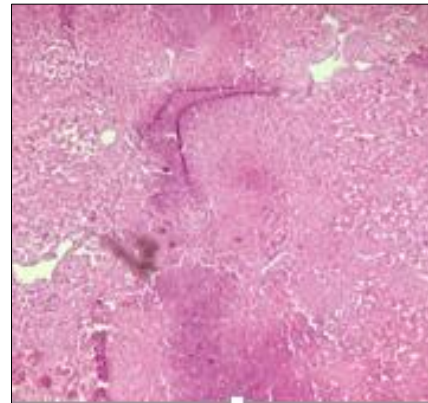


Fig 8: Severe degerative and necrotic changes with dialation of sinusoidal spaces

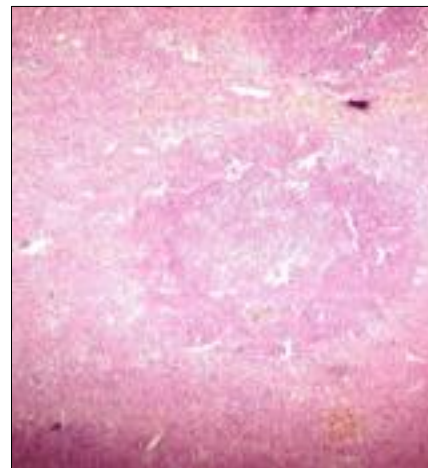


Fig 9: Formation of reactive germinal centers with necrotic changes in spleen

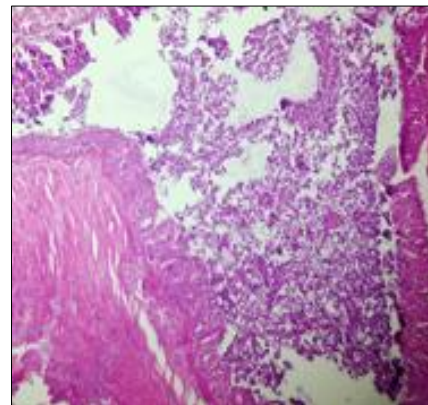


Fig 10: Denudation and clubbing of intestinal villi with necrotic debris and infiltration of MNCs in the lumen

Molecular diagnosis and differentiation of *S. gallinarum* and *S. pullorum* by multiplex PCR assay

Extraction of genomic DNA from culture

The genomic DNA of *Salmonella* spp. from positive overnight culture was extracted and its concentration was found to be 495-555 ng/μl. The DNA was used as template for PCR amplification of *Salmonella* spp. by targeting species specific I137_08605 and ratA genes.

Species specific Multiplex PCR assay

The gene I137_08605 specific for *S. gallinarum* and *S. pullorum* and gene ratA specific for *S. gallinarum* were targeted for multiplex PCR. PCR used 02 sets of primers (Xiong *et al.*, 2018) [35] (Table.1) to identify 08 field isolates. Only 03 of 08 *Salmonella* isolates produced a 290bp amplicon confirming *S. gallinarum* and/or *S. pullorum* (Fig. 12). These isolates also produced a 571bp amplicon confirming *S. gallinarum* (Fig. 13). Similar results were reported by Xiong *et al.*, (2018). This study found multiplex PCR targeting I137_08605 and ratA ROD genes 100% specific for detecting *S. gallinarum* and/or *S. pullorum* in outbreaks. Bioinformatics showed gene I137_08605 is in both *S. pullorum* and *S. gallinarum*, with a deletion in ratA ROD only in *S. pullorum*. *S. pullorum* and *S. gallinarum* can be accurately detected by targeting these genes (Carli *et al.*, 2001) [4]. Results showed a simple, accurate PCR assay for rapid identification of *S. gallinarum* and/or *S. pullorum*, useful for screening *Salmonella* infections (Newton, C. R. 1995) [19]. Nucleic acid techniques via species-specific multiplex PCR offer rapid identification and typing. These methods may replace cumbersome cultural, biochemical, and serological identification in the future (Zanetti *et al.*, 2019) [36]. Poor sample quality and delayed transport hinder cultural diagnosis. Nucleic acid techniques are the best alternative for rapid *Salmonella* spp. diagnosis (Xiong *et al.*, 2018)[35]. Molecular assays are highly specific and sensitive for identifying pathogens and differentiating closely related bacteria (Hu *et al.*, 2006). Studies show PCR detection of *Salmonella* serovars is more sensitive and faster than traditional methods (Oliveira *et al.*, 2002; Soria *et al.*, 2012) [20, 30]. A multiplex PCR assay was developed for

identifying and differentiating *Salmonella enterica* serovar *gallinarum* biovars Gallinarum and Pullorum. The unique gene I137_08605, present in biovars Gallinarum and Pullorum, is absent in other *Salmonella* serovars. Sequence analysis of ratA ROD in Gallinarum strains showed a deletion in Pullorum. The multiplex PCR used had 100% specificity. Our results indicated amplification of I137_08605 and ratA ROD can rapidly and reliably identify *S. gallinarum* and *S. pullorum*. This multiplex PCR may aid epidemiological investigations and serve as a potent diagnostic method in veterinary labs.

Nucleotide Sequencing

PCR products from 3 *Salmonella* positive samples from unvaccinated Durg flock were sequenced by Eurofins Pvt Ltd., Bangalore. Sequencing revealed a 571 bp sequence (Fig 11). Sequence analysis showed a complete 571 bp open reading frame (ORF). The Chhattisgarh *Salmonella enterica* subsp. *serovar gallinarum* isolate (Acc. No.Banklt2529837 BSeq#1 OL871236; Fig. 11) had 99.82% identity with abaetetuba strain (Acc. No. ATCC 35640), Brazil. Findings suggest Brazilian FT resurgence may stem from biosecurity failures, not evolution. The Chhattisgarh isolate also showed 99.82% identity with Gallinarum strain 9184 (Acc. No. CPO19035.1), Gallinarum strain 07Q015 (Acc. No. CPO77760.1), and Gallinarum strain 287/91 (Acc. No. AM933173.1). The isolate showed 99.82% identity with Javiana strain (Acc. No. CFSAN001992), Berta strain (Acc. No. CVM N17S334), Newlands strain (Acc. No. ZC-S1), London strain (Acc. No. CVM N17S347), enterica strain (Acc. No. CSFA1096), Newport strain (Acc. No. SAP18-8729), Muenchen strain (Acc. No. LG^[26]), Enteritidis strain (Acc. No. SE95), and Heidelberg strain (Acc. No. CVM N16S321). *S. Gallinarum* and *S. Pullorum* likely have low viability outside hosts due to impaired metabolic pathways (Thomson *et al.*, 2008; Feng *et al.*, 2013) ^[25, 7]. Thus, longdistance *S. Gallinarum* propagation is unlikely. Horizontal transmission is the main *S. Gallinarum* propagation route (Berchieri Junior *et al.*, 2001) ^[3]. Poultry exchange likely occurred between regions, introducing asymptomatic birds into healthy flocks.



Fig 11: Phylogenetic analysis of *Salmonella enterica* subsp. *Enterica serovar gallinarum* of Chhattisgarh under study along with other worldwide serovars on the basis of partial ratA ROD nucleotide sequences (571bp) available in GenBank.

Evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987) [23]. The bootstrap consensus tree from 1000 replicates represents the evolutionary history of the analyzed taxa [Felsenstein J. (1985) [6]]. The percentage of replicate trees clustering associated taxa in the bootstrap test (1000 replicates) is shown next to the branches. Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) [26], in base substitutions per site. This analysis involved 21 nucleotide sequences. Codon

positions included 1st+2nd+3rd+Noncoding. All ambiguous positions were removed (pairwise deletion).

The final dataset had 577 positions. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018) [15]. Red circle represents *Salmonella enterica* subsp. *Enterica serovar gallinarum* of Chhattisgarh, yellow circles represent different strains of *Salmonella enterica* subsp. *Enterica serovar gallinarum* from GenBank. Accession number allotted by GENBANK was Banklt2529837 BSeq#1 OL871236.

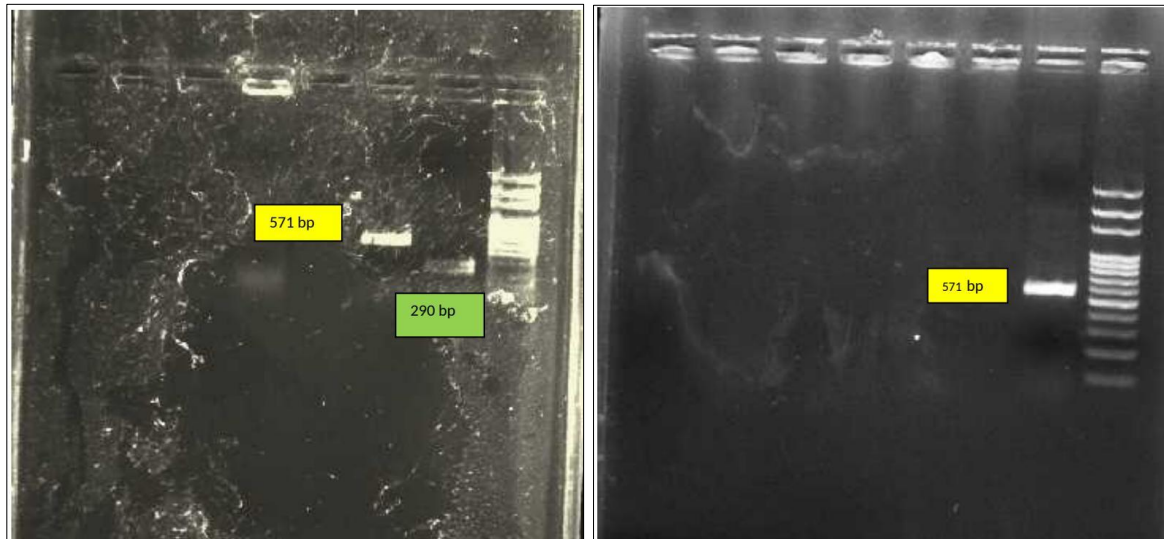


Fig 12: Amplification of *ratA* ROD isolates by multiplex PCR produced an amplicon of both 290bp and 571bp; **Fig 13:** Amplification of *ratA* ROD isolates by multiplex PCR produced an amplicon of 571bp.

In silico Protein Translation

Deduced protein sequence from translation of *ratA* ROD nucleotide sequence of *Salmonella enterica* subsp. *enterica serovar gallinarum* of Chhattisgarh using translate tool of Expasyserver.

TDMPNYLTCSANEMVAVLDDVYFNDDPVIKNADAKV
GEQVKMNIHSRNALNGIAIGNTDFTITMANGRRRDGL
TTGFTDTSNGEMQFDDVGYYAGQVYHGITDANGDA

TIILTQKKGVGLLTPLSIVPVNSYINTPVSRSVKFTVAT
SPDTAKAKMWGHMADTITVGDWTFERPKLAGEVSN
PLRTQDESN

In silico protein translation of *ratA* ROD nucleotide sequence of *Salmonella* Gallinrum isolates of Durg revealed a protein sequence of 190 amino acids.

Summary

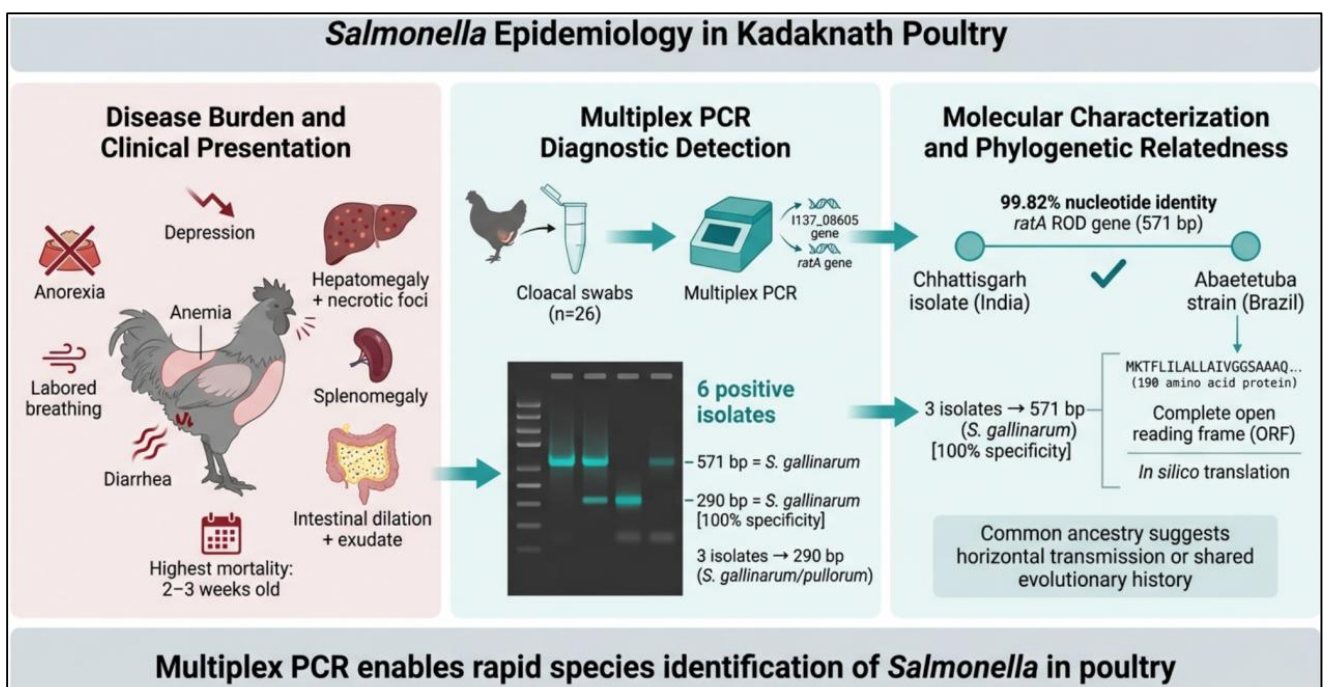


Fig 13: Clinical presentation, diagnostic detection, and molecular characterization of *Salmonella gallinarum* and *S. pullorum* isolates in Kadaknath chickens of the Chhattisgarh plains (Source: Grabstrack)

PCR amplification of clinical samples positive for *Salmonella Gallinarum* successfully produced a specific DNA amplicon with a size of approximately 571 bp. The phylogenetic analysis and molecular data from the present study revealed that the *Salmonella* strains identified in Durg and those previously documented in Abaetetuba, Brazil, likely shared a common evolutionary ancestor. *In silico* protein translation of the ratA ROD nucleotide sequences derived from the *Salmonella Gallinarum* isolates of Durg revealed a specific protein sequence consisting of 190 amino acids. Throughout the course of the present study, the ratA ROD gene of *S. Gallinarum* was targeted for detailed amplification and subsequent genetic sequencing. Further comprehensive investigation at the full genomic level will be extremely helpful in revealing subtle amino acid changes and mutations that might be indicative of the emergence of a new strain.

Conclusion

Affected chickens had hepatomegaly, splenomegaly, congestion, and necrotic foci in liver and spleen. Intestines and caeca showed dilation with yellowish exudate. Histopathology revealed congestion, hemorrhages, and necrosis in liver and spleen. Intestinal villi denudation, necrosis, MNC infiltration, and villi clubbing noted. PCR of *Salmonella Gallinarum* yielded a 571 bp amplicon. Durg's *Salmonella Gallinarum* sequence showed 99.82% similarity with Brazil's *Salmonella enterica* serovar Abaetetuba. Study indicated common ancestor for *Salmonella* Durg and Abaetetuba, Brazil. *In silico* protein translation of ratA ROD sequence revealed a 190 amino acid protein.

References

- Barrow PA, Jones MA, Smith AL, Wigley P. The long view: *Salmonella* – the last forty years. *Avian Pathol.* 2012;41:413-420.
- Blackall PJ, Soriano EV. Avian salmonellosis. In: *Diseases of Poultry*. 12th ed. Blackwell Publishing, Oxford; 2008. p. 789-803.
- Berchieri A Jr, Page KL, Smith AL, Barrow PA. *Salmonella enterica* serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent, disease-free carriage in chickens. *Infect Immun.* 2001;69(12):7873-7879.
- Carli KT, Unal CB, Caner V, Aysegul E. Detection of *Salmonellae* in chicken faeces by a combination of tetrathionate broth enrichment, capillary PCR, and capillary gel electrophoresis. *J Clin Microbiol.* 2001;39(5):1871-1876.
- Casagrande RA, Wouters AT, Wouters F, Pissetti C, Cardoso MR, Driemeier D. Fowl typhoid (*Salmonella Gallinarum*) outbreak in Japanese quail (*Coturnix coturnix japonica*). *Avian Dis.* 2014;58:491-494.
- Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution.* 1985;39:783-791.
- Feng Y, Johnston RN, Liu GR, Liu SL. Genomic comparison between *Salmonella Gallinarum* and Pullorum: differential pseudogene formation under common host restriction. *PLoS One.* 2013;8(3):e59427.
- Fotouh A, Gab-Allah MS, Tantawy AA, Soufy H, Nasir SM. Alterations of blood components in broiler chicks experimentally infected with *Salmonella Gallinarum*. *Global Veterinaria.* 2014;13:787-793.
- Freitas Neto OC, Arroyave W, Alessi AC, Fagliari JJ, Berchieri A Jr. Infection of commercial laying hens with *Salmonella Gallinarum*: clinical, anatomopathological and haematological studies. *Brazilian Journal of Poultry Science.* 2007;9:133-141.
- Garcia KO, Berchieri A, Santana AM, Alarcon MF, Freitas NC, Fagliari JJ. Experimental infection of commercial layers with wild or attenuated *Salmonella Gallinarum* mutant strains: anatomic pathology. *Brazilian Journal of Poultry Science.* 2013;15(2):91-103.
- Hafeji YA, Shah DH, Joshi BP, Roy A, Prajapati KS. Experimental pathology of field isolates of *Salmonella gallinarum* in chicken. *Indian Journal of Poultry Science.* 2001;36:338-340.
- Haider G, Chowdhury EH, Hossain M. Mode of vertical transmission of *Salmonella enterica* subsp. *enterica* serovar Pullorum in chickens. *African Journal of Microbiology Research.* 2014;8:1344-1351.
- Hendriksen RS, Vieira AR, Karlsmose S, et al. Global monitoring of *Salmonella* serovar distribution. *Foodborne Pathogens and Disease.* 2011;8(8):887-900.
- Hossain MS, Chowdhury EH, Islam MM, Haider MG, Hossain MM. Avian salmonellosis infection: isolation and identification of organisms and histopathological study. *Bangladesh Journal of Veterinary Medicine.* 2006;4(1):7-12.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution.* 2018;35:1547-1549.
- Islam MM, Haider MG, Chowdhury EH, Hossain MM. Seroprevalence and pathological study of *Salmonella* infections in layer chickens. *Bangladesh Journal of Veterinary Medicine.* 2006;4(2):79-85.
- Li Q, Wang X, Xia J, et al. *Salmonella*-containing vacuole development in avian cells and characteristics of *cigR* in *Salmonella enterica* serovar Pullorum replication within macrophages. *Veterinary Microbiology.* 2018;223:65-71.
- Long M, Li H, Deng W, et al. Disinfectant susceptibility of different serotypes isolated from chickens and egg production chains. *Journal of Applied Microbiology.* 2016;121(3):672-681.
- Newton CR. Nucleic acid substrates. In: *PCR: Essential Data*. New York: John Wiley and Sons; 1995. p. 24-26.
- Oliveira SD, Santos LR, Schuch DMT, Silva AB, Salle CTP, Canal CW. Detection and identification of *Salmonella* from poultry-related samples by PCR. *Veterinary Microbiology.* 2002;87:25-35.
- OIE. Fowl typhoid and Pullorum disease. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 7th ed. Paris: OIE; 2012. p. 1-14.
- Dubey P, Jolhe DK, Ghosh RC, Kumar V, Sonkusale PM. Prevalence, diagnosis and colony characterisation of salmonellosis in Kadaknath chickens. *Biological Forum.* 2026;18(3):74-80.
- Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution.* 1987;4:406-425.
- Saha AK, Sufian MA, Hossain MI, Hossain MM. Salmonellosis in layer chickens: pathological features and isolation of bacteria from ovaries and eggs. *Journal of Bangladesh Agricultural University.* 2012;10:61-67.
- Thomson NR, Clayton DJ, Windhorst D, et al. Comparative genome analysis of *Salmonella Enteritidis*

- PT4 and *Salmonella Gallinarum* 287/91. Genome Research. 2008;18(10):1624-1637.
26. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies using neighbor-joining method. Proceedings of the National Academy of Sciences USA. 2004;101:11030-11035.
27. Sannat C, Patyal A, Rawat N, *et al.* Characterization of *Salmonella gallinarum* outbreak in Raigarh, Chhattisgarh. Vet World. 2017;10:144-146.
28. Shivaprasad HL. Fowl typhoid and pullorum disease. Revue Scientifique et Technique Office International des Epizooties. 2000;19:405-424.
29. Soufy H, Gab-Allah MS, Tantawy AA, Fotouh A, Nasir SM. Pathogenesis of experimental *Salmonella Gallinarum* infection in broiler chicks. Egyptian Journal of Veterinary Science. 2016;47(2):117-131.
30. Soria MC, Soria MA, Bueno DJ. Comparison of culture methods and PCR assays for *Salmonella* detection. Poultry Science. 2012;91(3):616-626.
31. Syed HR, Khan MS, Hamayun K, Nazir A, Bhatti WM. Incidence and pathology of *Salmonella gallinarum* infection in chicken. Journal of Animal and Veterinary Advances. 2004;3:175-178.
32. Vugia DJ, Samuel M, Farley MM, *et al.* Invasive *Salmonella* infection in the United States. Clinical Infectious Diseases. 2004;38:S149-S156.
33. Wang X, Wang H, Li T, *et al.* Characterization of *Salmonella* spp. isolated from chickens in Central China. BMC Veterinary Research. 2020;16:299.
34. Wigley P, Hulme SD, Powers C, *et al.* Infection of reproductive tract and eggs with *Salmonella Pullorum*. Infection and Immunity. 2005;73:2986-2994.
35. Xiong D, Song L, *et al.* Identification and discrimination of *Salmonella enterica* serovar Gallinarum biovars Pullorum and Gallinarum based on multiplex PCR. Frontiers in Microbiology. 2018;9:1718.
36. Zanetti NS, Carli SD, Souza MN, *et al.* Molecular detection of *Salmonella Gallinarum* from poultry farms. Journal of Applied Poultry Research. 2019;28:1335-1341.
37. Zhang-Barber L, Turner AK, Barrow PA. Vaccination for control of *Salmonella* in poultry. Vaccine. 1999;17(20-21):2538-2545.