Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(1): 324-327

Received: 24-10-2020
Accepted: 05-12-2020

**Chandravathi T**
Ph. D Scholar, Department of Veterinary Pathology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

**Rama Devi V**
Professor and Head, Department of Veterinary Pathology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

**Satheesh K**
Principal, A.H. Polytechnic, Banavasi, Kurnool, Andhra Pradesh, India

**Ravi Kumar P**
Professor, Department of Veterinary Pharmacology and Toxicology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

**Sudhakar K**
Assistant Professor, Department of Animal Genetics and Breeding, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

**Muralidhar M**
Assistant Professor, Department of Animal Genetics and Breeding, College of Veterinary Science, Garividi, Andhra Pradesh, India

**Corresponding Author:**
**Chandravathi T**
Ph. D Scholar, Department of Veterinary Pathology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

# Pathological and molecular diagnosis of spontaneous cases of complicated infectious coryza in commercial chicken

**Chandravathi T, Rama Devi V, Satheesh K, Ravi Kumar P, Sudhakar K and Muralidhar M**

**DOI:** https://doi.org/10.22271/j.ento.2021.v9.i1e.8163

**Abstract**
Respiratory diseases have emerged as a great challenge to poultry industry and result in severe economic losses. Infectious coryza caused by *Avibacterium paragallinarum* (*A. paragallinarum*) affects upper respiratory tract. In the present study the pathological and molecular diagnosis of infectious coryza was carried out in commercial chicken from the Andhra Pradesh region. Samples were collected from the suspected birds of different poultry farms. Out of 20 pooled samples collected, 18 (90%) samples were found positive for infectious coryza by PCR, in which 2 samples were also found positive for *E. coli* on bacteriological examination. The affected birds showed respiratory symptoms like open mouth breathing, coughing, sneezing. Unilateral to bilateral edema of wattle and combs were observed in severely affected birds. In infectious coryza sinusitis, tracheitis, laryngitis, mild airsacculitis and pneumonia were noticed. In complicated cases of *A. paragallinarum* and *E. coli* severe sinusitis, tracheitis, laryngitis, airsacculitis, pneumonia, perihepatitis and pericarditis were noticed. From this study, it is concluded that PCR is an early, rapid, accurate and highly sensitive diagnostic technique which replace the conventional cultural examination and can be used as early diagnostic tool to prevent the economic losses associated with the disease.

**Keywords:** *A. paragallinarum*, chicken, infectious coryza, pathology, molecular diagnosis

## Introduction
Respiratory diseases have emerged as a great challenge to poultry industry. Infectious coryza is a respiratory disease of chicken caused by the bacterium, *Avibacterium paragallinarum* affecting upper respiratory tract with involvement of nasal passages, infraorbital and paranasal sinuses [1]. *A. paragallinarum*, previously known as *Haemophilus paragallinarum* [2] often gets complicated by the presence of other pathogens such as *M. gallisepticum*, *M. synoviae*, *E. coli*, *P. multocida* and viral pathogens like IBV, ILTV and fowl pox virus [3]. The economic losses associated with infectious coryza result from poor growth performance in broilers, marked reduction in egg production in layers and increased culling rates in meat chicken. Chronically infected birds or recovered healthy birds act as reservoirs of infection in a population and makes the disease endemic in an area. The disease is recognized as a cause of significant loss to the poultry industry all over the world. For reducing the economic losses associated with this disease, early, rapid and accurate diagnosis is essential. The conventional diagnosis of infectious coryza is based on clinical signs, demonstration of satellite colonies by cultural examination and confirmation by biochemical tests. But the factors like simultaneous occurrence of combined respiratory infections, occurrence of nicotinamide adenine dinucleotide (NAD) independent strains, overgrowth of fast growing bacteria which are masking the growth of *A. paragallinarum*, requirement of special media for culturing, presence of different biovars etc. make the confirmatory diagnosis difficult. Hence, nucleic acid based techniques are the best alternative tools in the easy and rapid confirmatory diagnosis. *E. coli* being ubiquitous organism in poultry production, any stress to the respiratory tract of chicken creates a climate for colonization *E. coli* in the respiratory tract. Keeping this in view, in the present study pathological and molecular diagnosis of infectious coryza was carried out in commercial chicken.

## Materials and Methods

The present study was carried out at department of Veterinary Pathology, NTR College of Veterinay Science, Gannavaram. Twenty commercial poultry flocks suspected for the infectious coryza were visited and pooled samples consisting of ocular, nasal and tracheal swabs were collected from each flock for diagnosis. A total of 20 pooled samples were collected aseptically and soaked in phosphate buffered saline and transported to laboratory on ice and stored at -20°C for molecular diagnosis. The collected swabs were used for extraction of DNA from *A. paragallinarum* as per the protocol described by earlier author with certain modifications [4]. For PCR screening of the samples the specific primers N1 (5' TGA GGG TAG TCT TGC ACG CGA AT 3') and R1 (5' CAA GGT ATC GAT CGT CTC TCT ACT 3') of HPG-2 gene described by Chen *et al.,* (1996) were used that amplify 500 bp fragment of *A. paragallinarum* [5]. The DNA extracted from the infectious coryza killed vaccine was used as positive control in the present study. PCR was carried out as per standardized cycling conditions consisting of an initial denaturation at 94°C for 3min followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 62°C for 60 sec, extension at 72°C for 45 sec and a final extension at 72°C for 5 min. The amplified products were subjected to agarose gel electrophoresis. Nasal swabs collected aseptically from suspected birds and were soaked in 30% glycerol-phosphate buffered saline and were revived in the nutrient broth and BHI broth. They were further streaked on nutrient agar, and single colonies from the nutrient agar were restreaked on EMB, MacConkey and Blood agar plates for enrichment. Bacteria Identification was based on the colony morphology and Gram's staining [6]. Detailed post mortem examination of suspected birds was carried out and gross lesions in different organs were recorded and pieces of the tissues were collected in 10% formalin for histopathological studies.

## Results and discussion

In the present study, suspected birds for infectious coryza were collected from different poultry flocks and pathological and molecular diagnosis of *A. paragallinarum* was carried out.

### Molecular diagnosis

In the present study, DNA extracted from 20 pooled samples was used in the PCR assay for molecular detection of *A. paragallinarum*. PCR primers targeting HPG-2gene of *A. paragallinarum* amplified an expected size of 500bp amplicon that confirmed the identity of *A. paragallinarum* in 18(90%) samples (Fig.1). Chen *et al.* (1996) developed a PCR test (HP-2) for rapid and easy diagnosis of infectious coryza infections directly from the sinus swabs and recommended PCR as a confirmatory test than culture studies [5]. The PCR results are in conformity to previous results, the authors used PCR assay for detection of *A. paragallinarum* from chicken infected with infectious coryza [7, 8]. Anjaneya *et al.* (2014) detected *A. paragallinarum* directly from the nasal and sinus swabs using HP-2 PCR developed by Chen *et al.* (1996) and stated that PCR techniques are the best alternative tools in the easy and rapid confirmatory diagnosis of *A.paragallinarum* from field samples[2].

Further, on bacterial studies, the presence *E.coli* along with the *A.paragallinarum* was identified in 2 samples. In previous studies also some of the the authors mentioned that *A. paragallinarum* is complicated with the fowl cholera [9], *Mycoplasma gallisepticum* [10] and *E. coli* [11]. Dwivedi *et al.* (2018) also isolated *E. coli* and *Klebsiella spp., Salmonella spp.,* and one and Pseudomonas spp. from the *A. paragallinarum* infected birds [11].

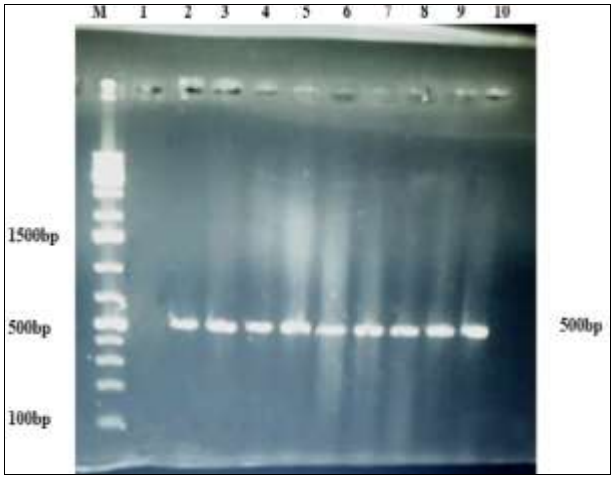


**Fig 1:** Agar gel electrophoresis of amplified PCR products of HPG gene of *A. paragallinarum* (500 BP). Lane M- Ladder100bp, Lane 1-Negative control, Lane 2 to9-DNA samples, Lane 10-positive control

### Pathology

The prominent symptoms observed are serous to mucoid nasal discharge, facial edema and swelling and conjunctivitis. Respiratory symptoms like open mouth breathing, coughing, sneezing were noticed. Unilateral to bilateral edema of wattle and combs were observed in some affected birds. These symptoms were also observed by the previous authors [2, 12, 13]. In a study Dwivedi *et al.* (2018) noticed signs open mouth breathing, coughing, sneezing, conjunctivitis, occulonasal discharge, oedema of face and wattles which were similar to our study [11]. Our results were also akin with the results of Droual *et al.* (1990) who recorded nasal discharge, swelling of the sinuses, severe foamy lacrimation and conjunctivitis in an outbreak of infectious coryza [12].

Grossly, the affected bird's revealed swelling around eyes (Fig.2), facial swelling (Fig.3), conjunctivitis and a serous to mucoid or purulent nasal discharge from nostrils and sinuses in the initial stages. As the disease advances caseous exudates were seen in the paranasal and infraorbital sinuses (Fig.4). Few birds showed congestion of trachea, edema and congestion of lungs and thickened air sacs (Fig.5). The lesions found in the present study are in accordance with the lesions reported by previous authors [2, 12, 13]. These observations were similar to Blackall and Soriano (2005), who also observed swelling of eyes and face, conjunctivitis, mucus discharge from nostrils and caseous material in the paranasal and infraorbital sinuses in the birds infected by *A. paragallinarum* [2]. The lesions in the trachea and lungs are in accordance with the lesions described by Droual *et al.* (1990) and Hoerr *et al.* (1994), the authors noticed congestion of trachea, edema and congestion of lungs and thickened air sacs in birds infected by *A. paragallinarum* [12, 13].

In concurrent infection of *A. paragallinarum* and avian pathogenic *E. coli* the lesions observed were conjunctivitis, sinusitis, thickened air sacs and fibrinous exudates on the pericardium and liver. These similar lesions were also reported by Christensen *et al.* (2002) Dwivedi *et al.* (2018) in mixed infection of *A. paragallinarum* and *E. coli* [11, 14]. Dwivedi *et al.* (2018) noticed conjunctivitis, swollen sticky

eyelids, swollen face and sinuses with fibrinous exudates, congested lungs and mild to moderate deposition of fibrinous material over the heart, liver and air sacs in concurrent infection of *A. paragallinarum* and *E. coli* [14].

Microscopically, the nasal sinuses revealed thickened lamina propria and hypertrophy of mucosal glands initially and later on epithelial cell degeneration and infiltration of mononuclear cells and heterophils (Fig.6) as found by Haunshi *et al.* (2006) in *A. paragallinarum* infection [15]. The laryngeal mucosa was severely congested along with epithelial cell degeneration. Trachea showed congestion of mucosa, desquamated epithelial cells, hypertrophy of mucosal glands and mononuclear cell infiltration. Congestion, degeneration of the epithelium and infiltration of mononuclear cells and heterophils in the submucosa were noticed in the air sacs. Lungs showed congestion, mild haemorrhages, thickening of interlobular septa and infiltration of mononuclear cells in the alveolar walls (Fig.7). Lesions like congestion, desquamated epithelial cells and mononuclear cell infiltration in the trachea and air sacs and congestion, haemorrhages, thickening of interlobular septa and infiltration of mononuclear cells in the alveolar walls in lungs are akin to the reports of Anjaneya *et al.* (2013) and Dwivedi *et al.* (2018) studies [3, 11]. Anjaneya *et al.* (2013) observed thickened paranasal sinuses with infiltration of mononuclear cells and heterophils, desquamated epithelial cells and cellular infiltration in the trachea and air sacs and thickening of inter bronchial area and congestion in lungs in birds infected by *A. paragallinarum* [3].

In concurrent infections of *A. paragallinarum* and avian pathogenic *E. coli*, in addition to the above respiratory lesions, mild fibrinous exudation and infiltration of mononuclear cells and heterophils were observed in the pericardium and liver (Fig.8 and 9). Similar changes were described by Dwivedi *et al.* (2018) and Christensen *et al.* (2002) in *A. paragallinarum* and *E. coli* concurrent infections [11, 14]. In simple infection of *A. paragallinarum* the lesions were mild and in complicated cases with *E. coli* the lesions were more severe with involvement other liver and heart.

## Conclusion

PCR is a rapid and highly sensitive diagnostic technique that can substitute conventional cultural examination of *A. paragallinarum.* In infectious coryza sinusitis, tracheitis, laryngitis, mild airsacculitis and pneumonia were noticed and in complicated cases of *E. coli* along with respiratory lesions, perihepatitis and pericarditis were also noticed.

**Fig 2:** Bird showing swelling around eyes

**Fig 3:** Bird showing swelling of face

**Fig 4:** Bird showing caseous exudates in sinus

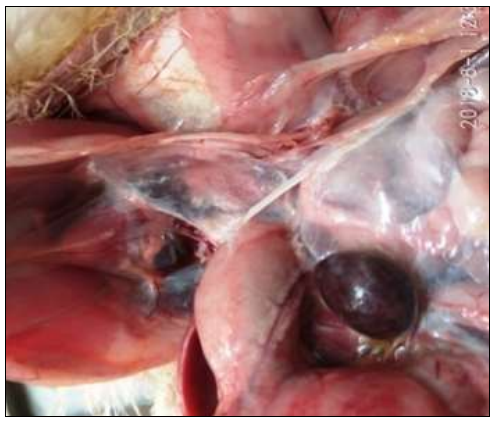

**Fig 5:** Bird showing thickened air sacs

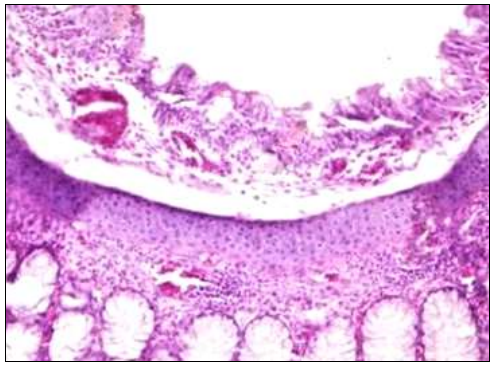

**Fig 6:** Sinus showing congestion and epithelial cell desquamation and infiltration of mononuclear cells and heterophils H&Ex100

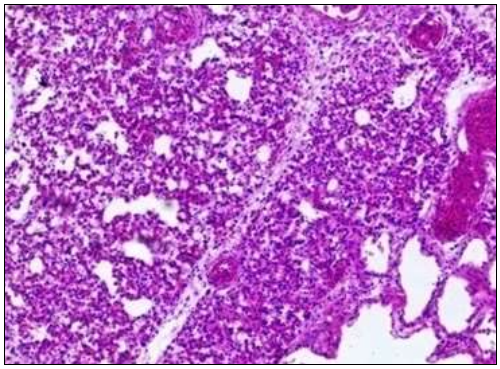

**Fig 7:** Lung showing congestion and mild diffuse haemorrhages, thickening of interlobular septa and infiltration of mononuclear cells in the parabronchi H&Ex100

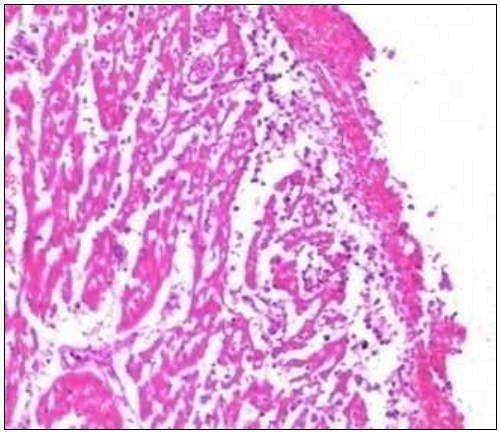

**Fig 8:** Heart showing mild fibrinous exudation along with infiltration of mononuclear cells and heterophils in epicardium H&E x100

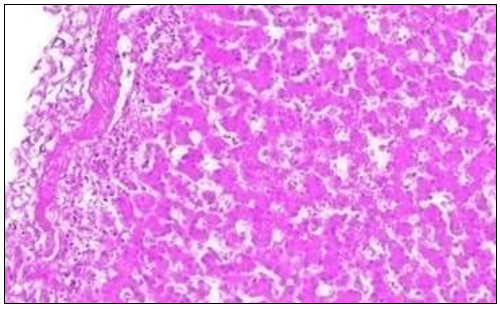

**Fig 9.** Liver showing mild fibrinous exuadate along with mononuclear cells and heterophils on surface H&E x100

**Acknowledgement**
The authors are grateful to the College of Veterinary Science, Gannavaram, SVVU, and Andhra Pradesh for providing necessary facilities to carry out the research work.

**References**

1. Ali M, Hossain MS, Akter S, Khan Mahna, Hossain MM. Pathogenesis of Infectious Coryza in chickens (Gallus gallus) by *Avibacterium paragallinarum* in isolates of Bangladesh. The Agriculturists 2013;11(1):39-46
2. Blackall PJ, Soriano VE. Infectious coryza and related bacterial infections in Diseases of Poultry. Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE (ed) 12th edn Iowa State University Press, Towa, USA 2012, 789-803.
3. Anjaneya Singh SD, Dhama K, Gowthaman V, Chawak MM. Pathogenicity study of field isolates of *Avibacterium paragallinarum* in experimentally infected birds. Indian Journal of Veterinary Pathology 2013;37(1):13-7.
4. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press. New York 2001, 3.
5. Chen X, Miflin JK, Zhang P, Blackall PJ. Development and application of DNA probes and PCR tests for Haemophilus paragallinarum. Avian Diseases 1996;40:398-407.
6. Sawata A, Kume K. Relationship between virulence and morphological or serological properties of variants dissociated from serotype 1 Haemophilus paragallinarum strains. Journal of Clinical Microbiology 1983;18:49-55
7. Kaur J, Banga HS, Sood NK, Gupta K, Singh A. Pathological studies on naturally occurring infectious coryza in chickens. Indian Journal of Animal Science 2005;75:209-211.
8. Byarugaba DK, Minga UM, Gwakisa PS, Katunguka-Rwakishaya E, Bisgaard M, Olsen JE. Investigations of the occurrence of *Avibacterium paragallinarum* Infections in Uganda. Avian Disease 2007;51(2):534-9
9. Tongaonkar S, Deshmukh S, Blackall PJ. Characterization of Indian isolates of Haemophilus paragallinarum. In Proceedings of the 51st Western Poultry Disease Conference, Peurto Vallajarta, Mexico 2002P, 58.
10. Kato K. Infectious coryza of chickens. V. Influence of *Mycoplasma gallisepticum* infection on chicken infected with *Haemophilus gallinarum*. National Institute of Animal Health Quarterly 1965;5(4):183.
11. Dwivedi S, Swamy M, Dubey A, Verma Y, Singh AP. Infectious coryza in birds complicated by other bacterial infections. Indian Journal of Veterinary Pathology 2018;42(4):271-5.
12. Droual R, Bickford AA, Charlton BR, Kuney DR. Investigation of problems associated with intramuscular breast injection of oil-adjuvanted killed vaccines in chickens. Avian Diseases 1990, 473-8.
13. Hoerr FJ, Putnam M, Rowe-Rossmanith S, Cowart W, Martin J. Case report: Infectious coryza in broiler chickens in Alabama. InWestern Poultry Disease Conference (USA) 1994.
14. Christensen H, Dziva F, Olsen JE, Bisgaard M. Genotypic heterogeneity of *Pasteurella gallinarum* as shown by ribotyping and 16S rRNA sequencing. Avian Pathology 2002;31(6):603-10.
15. Haunshi S, Dutta B, Saxena SC. An outbreak of infectious coryza in Vanaraja poultry of Meghalaya. Indian Journal of Veterinary Pathology 2006;30(1):55.