MIXED INFECTION OF INFECTIOUS LARYNGOTRACHEITIS AND *ORNITHOBACTRIUM RHINOTRACHEALE* IN LAYER CHICKEN FLOCKS IN NAMAKKAL REGION OF TAMIL NADU

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ABSTRACT

Among the respiratory diseases of poultry, infectious laryngotracheitis (ILT) is one of the most economically important diseases, impairing poultry health and welfare by causing morbidity, mortality, reduced egg production, and decreased meat yield. Further secondary complications increases the severity of the disease. *Ornithobacterium rhinotracheale* (ORT) is an emerging respiratory pathogen that causes primary or opportunistic infection leading to eye swelling and conjunctivitis. In most of the occasions ILT is complicated with secondary pathogens leading to severe disease, conjunctivitis, and prolonged course of disease. No much information is available regarding the co-infection of ILT and its complicating pathogens. Hence this present study is undertaken on incidence of mixed infection of infectious laryngotracheitis and *Ornithobactrium rhinotracheale* in layer chicken flocks in Namakkal district of Tamil Nadu. A diagnostic investigation was carried out in Namakkal region of Tamil Nadu during March 2023 to November 2024 from seven commercial layer chicken flocks aged between 6-13 weeks having the incidences of respiratory symptoms suggestive of infectious laryngotracheitis. Necropsy examination was carried out, clinical samples were collected and subjected to molecular diagnosis of ILT and ORT. The results of laboratory investigation revealed that all the seven flocks were positive for ILT and three out of seven commercial layer flocks were also found positive for ORT by PCR, which conformed the co-circulation of ILTV and ORT in layer chicken flocks of Namakkal.

Keywords: Infectious laryngotracheitis, *Ornithobactrium rhinotracheale*, mixed infection, grower chicken

Introduction

The poultry industry is one of the fastest-growing sectors of animal agriculture and plays a vital role in ensuring food security, nutritional security, and economic development. India is among the world's leading producers of eggs and poultry meat, with the industry contributing significantly to the agricultural economy and providing livelihoods to millions of people. In recent years a growing demand for chicken meat and eggs resulted in significant expansion of the poultry population in India (Doley *et al.,* 2025). However, intensive production systems with high stocking density, continuous bird movement, and environmental stress have increased the risk of infectious disease outbreaks, especially those affecting the respiratory tract. Respiratory diseases are among the most economically important health problems in poultry because they result in increased mortality, reduced growth, poor feed conversion efficiency, decreased egg production, and increased treatment costs. Among different infectious diseases of poultry respiratory tract infections cause major economic loss in poultry industry worldwide causing individual disease or a combination of diseases (Yashpal *et al*., 2004).

Infectious laryngotracheitis (ILT) is an acute, highly contagious respiratory disease of chickens caused by Infectious laryngotracheitis virus (ILTV), a member of the family *Herpesviridae*. The disease affects chickens of all ages, although severe outbreaks are more frequently observed in commercial layer and breeder flocks. Clinically, ILT is characterized by gasping, coughing, dyspnoea, conjunctivitis, nasal discharge, and expectoration of blood-stained mucus. Severe cases often exhibit haemorrhagic tracheitis with blood clots and fibrinonecrotic exudates obstructing the tracheal lumen. The disease causes substantial economic losses due to increased mortality, decreased egg production, poor body weight gain, and reduced flock performance (Mo and Mo, 2025). An important feature of ILTV is its ability to establish latent infection in recovered birds, which may subsequently act as reservoirs and contribute to the persistence and spread of infection within poultry populations.

Respiratory diseases in poultry are frequently multifactorial, involving simultaneous infections with viral and bacterial pathogens. Viral infection damages the respiratory epithelium, impairs mucociliary clearance, and predisposes birds to secondary bacterial infections, resulting in increased disease severity and prolonged recovery. Among the emerging bacterial respiratory pathogens, *Ornithobacterium rhinotracheale* (ORT) has gained considerable importance in commercial poultry production worldwide. ORT is associated with respiratory disease characterized by pneumonia, airsacculitis, conjunctivitis, facial swelling, nasal discharge, reduced weight gain, decreased egg production, and increased mortality (Barbosa *et al.,* 2019). Although ORT can act as a primary pathogen, it is more commonly isolated as an opportunistic organism associated with other respiratory pathogens, thereby contributing to the avian respiratory disease complex.

The interaction between ILTV and secondary bacterial pathogens often results in more severe clinical disease than

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infection with either pathogen alone. Co-infections increase tissue damage, prolong the clinical course, and complicate diagnosis and disease control. Previous studies have documented concurrent infections of ILTV with pathogens such as *Mycoplasma gallisepticum*, *Avibacterium paragallinarum*, and fowlpox virus. However, published information regarding concurrent infection of ILTV and *Ornithobacterium rhinotracheale* under Indian field conditions remains limited. Considering the high density of commercial poultry farms in the Namakkal region, surveillance of respiratory pathogens and their co-infections is essential for effective disease management and prevention.

Molecular diagnostic techniques, particularly polymerase chain reaction (PCR), have become valuable tools for the rapid and accurate detection of respiratory pathogens in poultry. PCR provides high sensitivity and specificity, enabling early diagnosis and differentiation of pathogens that produce similar clinical signs. In most of the occasions ILT is complicated with secondary pathogens leading to severe disease, conjunctivitis, and prolonged course of disease (Rodrigo *et al.,* 2016). No much information is available regarding the co-infection of ILT and its complicating pathogens. Therefore, the present study was undertaken to investigate the occurrence of concurrent infection of Infectious laryngotracheitis virus and *Ornithobacterium rhinotracheale* in commercial layer chicken flocks in the Namakkal region of Tamil Nadu using PCR-based diagnosis, and to describe the associated clinical manifestations, pathological lesions, and epidemiological significance of this mixed infection.

Materials and Methods

Disease investigation and sample collection

A diagnostic investigation was carried out in Namakkal region of Tamil Nadu during March 2023 to November 2024 from seven commercial layer chicken flocks aged between 6-13 weeks having the incidences of respiratory symptoms suggestive of infectious laryngotracheitis. Ten recently dead birds/farm were brought to the Poultry disease diagnosis and surveillance laboratory for pathological, and molecular diagnostic studies. Post-mortem examination was performed and tracheal samples were collected for diagnostic studies.

Nucleic acid extraction and PCR

Viral DNA from intestinal tissues was extracted by using by using Wizard Genomic DNA purification kit (Promega, USA) as per the standard instruction of the manufacturer. The samples were screened for glycoprotein-G gene (US4 gene) of ILTV by polymerase chain reaction (PCR) by using primers viz., ILT p32 U2-CTA CGT GCT GGG CTC TAAT CC and ILT p32 L2-AAA CTC TCG GGT GGC TAC TGC (amplicon size 588 bp) with an with an initial denaturation at 95 °C for 15 min, followed by 35 cycles at 95 °C for 1 min, 61 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. Finally two micro litres of PCR products were analysed by gel electrophoresis in 1.5 % agarose. Further ILTV positive flocks were subjected to PCR diagnosis of ORT by targeting 16S gene using primers viz. 16S F 5'–GAGAATTAATTTACGGATTAAG-3' and 16S R 5'–TTCGCTTGGTCTCCGAAGAT-3' (amplicon size 784 bp) with an initial denaturation at 95 °C for 15 min, followed by 35 cycles at 94 °C for 30 sec, 52 °C for 1 min, 72 °C for 1.5 min, and a final extension at 72 °C for 10 min as per the methods described by (Murthy *et al.,*2008 and Gowthaman *et al.,* 2015).

Results and Discussion

The results of laboratory investigation revealed that all the seven flocks were positive for ILT and three out of seven commercial layer flocks were also found positive for ORT by PCR, which conformed the co-circulation of ILT and ORT. The intensity of clinical signs were more severe in ORT co-infected flocks. The mortality of the affected flocks ranged between 0.5-2.0% and the ORT co-infected flocks had higher mortality.

Clinical signs

The affected commercial layer chicken flocks exhibited characteristic clinical signs ofILT, which were markedly more severe in birds concurrently infected with *Ornithobacterium rhinotracheale* (ORT). The disease was characterized by respiratory distress with dyspnoea, gasping, coughing, sneezing, and pronounced pump-handle respiration. Serous to mucoid nasal discharge, swelling of the paranasal sinuses, and conjunctivitis with swollen eyelids and excessive watery ocular discharge. In severely affected birds, the eyelids became adhered due to dried ocular exudates. The birds appeared dull, depressed, and lethargic, with reduced feed intake and decreased activity. The clinical course exceeded 10 days in co-infected flocks, indicating prolonged illness. Mortality ranged from 0.5% to 2.0%, with higher mortality recorded in ILT–ORT co-infected flocks, suggesting that concurrent ORT infection aggravated the severity of ILT.

Gross lesions

Necropsy examination revealed characteristic respiratory lesions. The trachea showed severe haemorrhagic tracheitis with marked congestion, blood-stained mucus, blood clots, and fibrinonecrotic exudates occluding the tracheal lumen. Whitish to yellowish fibrinopurulent material and diphtheritic plaques adhered firmly to the tracheal mucosa, causing narrowing of the airway. Pulmonary lesions included marked congestion, consolidation, and red hepatization, indicative of severe pneumonia. Generalized congestion of visceral organs, particularly the spleen, liver, and mesentery, was consistently observed. These lesions were more extensive in birds with concurrent ILT and ORT infection than in birds infected with ILT alone. The severe tracheal and pulmonary lesions, together with the prolonged clinical course and increased mortality, indicate that ORT co-infection significantly exacerbated the pathological manifestations of infectious laryngotracheitis in commercial layer chickens.

Molecular diagnosis

DNA was extracted from all the7 flocks with clinical disease suggestive of ILT and screened for p32 gene of ILT. ILT was found positive all the seven flocks screened. The positive samples showed amplification at 588 bp of p32 gene. The ILT positive samples were further subjected to molecular confirmation of *Ornithobactrium rhinotracheale* by PCR, which revealed that three out of seven ILT positive flocks were also found positive for *Ornithobactrium rhinotracheale* confirmed the co-circulation of ILT and ORT.

In this study, totally seven commercial layer poultry flocks aged between 6-13 weeks were investigated with the history

suggestive of ILT and all seven flocks were found positive for ILT and three flocks were found positive for ORT along with (42.8%) concurrent infections of ILT. Further ILT and ORT co-infected flocks exhibited severe clinical signs, higher mortality with prolonged course of disease. Previous studies of (Couto *et al.,*2015) also reported the natural concurrent infections such as *Mycoplasma gallisepticum* and *Avibacterium paragallinarum* associated with infectious laryngotracheitis in layer chickens.Song *et al*., (2021) observed simultaneous detection of infectious laryngotracheitis virus, fowl pox virus and reticuloendotheliosis virus in chicken specimens. The results in the present study agree with the fact that etiology of ILT in poultry is very complex and often involves more than one pathogen at a time during its clinical progression. The above results clearly indicate that co-infections play a pivotal role in the clinical course of ILT respiratory disease in poultry. Furthermore (Murthy *et al.,* 2008) reported the isolation of *O. rhinotracheale* either singly or concurrently with other bacteria, such as *E. coli* and *A. paragallinarum* in the respiratory disease complex of poultry. It indicates the possible etiological role of ORT in ILT disease complex in poultry. The symptoms of nasal discharge, coughing, gasping, sneezing, laboured breathing, expectoration of blood stained mucus and pump hand respiration observed in this study are in agreement with the earlier reports of (Srinivasan *et al.,* 2012; Gowthaman *et al*. 2014). Infected birds were lethargic and had swollen eyelids, reddened conjunctiva and watery eyes. In severe cases, eyelids were adhered with dry, crusty, ocular discharge sticking to the eyelids and skin around the eye. This clinical signs was also reported by (Tahseen, 2010). In our study PCR was used to diagnose the ILT and ORT. Williams *et al.* (1992) reported that PCR facilitates the amplification of target sequences and offers several advantages over other diagnostic methods, virus need not be viable prior to their detection by PCR, the technique possesses high sensitivity and specificity capable of detecting target DNA in the complex mixture so the state of the tissue and/or presence of other viruses or bacteria do not interfere with the final result and it is rapid and versatile.

Conclusions

The present study reveals that ILT is one of the important respiratory disease in Namakkal, region. Co-infections of *Ornithobactrium rhinotracheale* also present with ILT were observed. It proves that synergism has been demonstrated between ILT and *Ornithobactrium rhinotracheale*. In ILT outbreak, due attention is needed to detect and control the co-infections in order to avoid more economic loss which could have aggravated the pathology of ILT.

Conflicts of interest

The authors declare that they have no competing interests.

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