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Occurrence and pathology of infectious laryngotracheitis (ILT) in commercial layer chicken

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Abstract
Infectious laryngotracheitis (ILT) is a viral disease of the respiratory tract of chicken that occur in areas where there is intensive poultry production and responsible for severe economic losses. The present study was aimed to investigate the occurrence and pathology of infectious laryngotracheitis in commercial layer chicken in Namakkal, Tami Nadu and Puducherry. Out 62 commercial layer farms investigated in the present study, 40 (64.5%) were identified as positive for ILT. The occurrence of ILT varied from 4 to 70 weeks of age. Birds between the age group of 10 to 20 weeks were more affected and the mortality rate ranged from 1% to 17%. In layers, the mortality varied from 1% to 6% with production loss of 12%. The disease was prevalent throughout the year and the course of the disease extended for 30 days. Clinically, dullness, reduced feed and water intake, respiratory distress gasping, coughing up blood or blood tinged mucus and pump handle-type respiration was observed. Grossly trachea revealed catarrhal, haemorrahgic and/or necrotic changes. Histopathological examination revealed degeneration of tracheal epithelium, hyperplasia with syncytial formation, complete necrosis and infiltration by inflammatory cells. Occasionally, intranuclear inclusion bodies in the epithelial cells, cystic dilation of submucosal gland and lymphoid aggregations were noticed.

Keywords: Gross and histopathology, ILT, layer chicken, occurrence

Introduction
Poultry production in India has taken a quantum leap in the last four decades, emerging from an unscientific farming practice to commercial production system with state-of-the art technological interventions. The best fed and housed poultry stock with the best genetic potential will not grow and produce efficiently if they become diseased or infested with parasites. Selective breeding policy, lack of bio-security, increased intensity of rearing and improper vaccination has led to the increased incidence of diseases in layers. Among them, diseases affecting the respiratory and digestive systems are most common, accounting for nearly 70% of all the cases seen in a diagnostic laboratory or in the poultry farms [1].

Among the prevailing respiratory diseases, avian infectious laryngotracheitis (ILT) is one of the important contagious viral respiratory diseases that cause significant economic losses in the poultry industry due to increased mortality, decreased growth rate and lower egg production [2]. The disease mostly affect the upper respiratory tract of chickens and is caused by infectious laryngotracheitis virus (ILTV; *Gallid herpesvirus* 1), a member of the sub-family *Alpha herpes virinae* (Genus *Iltovirus)*. This virus is only transmitted horizontally and primarily infects the conjunctiva and the tracheal mucosa [3].

The target organ system for ILTV infection and disease is the respiratory tract. The epithelium of the trachea and larynx is always affected, whilst other mucous membranes such as the conjunctiva, as well as respiratory sinuses, air sacs and lung tissue may also become infected periodically [4]. Clinical signs generally appear 6-12 days following natural exposure and include nasal discharge, moist rales, conjunctivitis and labored breathing with expectoration of blood-stained mucus in severe cases [5].

Pathological changes are restricted to the upper respiratory tract, primarily trachea characterized by mucoid and haemorrhagic to necrotic tracheitis. Microscopically, lose cilia, degeneration of epithelium, edema, haemorrhage, hyperplasia of epithelial cells with the formation of syncytia and infiltration by lymphocytes, histiocytes, and plasma cells are observed. Presence of intra nuclear inclusion bodies in the epithelial cells of the trachea, often considered pathognomonic is rarely present [5].

The incidence of ILT is on the rise in many parts of the world, which may be due to the use of modified live ILTV vaccines developed by serial passage of its virulent strain in cell cultures or in embryonated chicken eggs. Nevertheless, these vaccines are capable of regaining virulence after serial bird to bird passages and establish latent or clinical infections [6]. Recent studies revealed spontaneous and natural recombination between attenuated vaccines and field strains of ILTV leading to the emergence of its novel variants that can cause widespread disease [7]. Considering the above facts, the present study was aimed to investigate the occurrence and pathology of infectious laryngotracheitis in commercial layer chicken.

Materials and Methods

Study area: Samples for the present study were collected from the various commercial layer farms located in and around Namakkal (Tamil Nadu), India and also from poultry carcasses referred to the Department of Veterinary Pathology, RIVER, Puducherry, India. Samples were collected from those birds which had lesions suggestive of ILT. A total of 105 samples (100 from Namakkal and 5 from Puducherry) were collected. Namakkal District in Tamil Nadu, India was chosen for the study because this area has made a remarkable progress in commercial layer farming with 50 million layer stock with a production of 40 million eggs per day in about 550 poultry farms contributing 80% to the egg production in Tamil Nadu, India and accounting for 90% of the total egg exports from the country.

Observations: The details of the farms such as total flock size (Chick, Grower and Layer), system of rearing, affected age group, mortality, morbidity, production loss and concurrent infection were collected using a questionnaire. In this present study, a total of 62 commercial layer farms were investigated. The flock size of the poultry farms varied from 18,000 to 2, 50,000 birds. The strain of birds maintained included BV300, Lohmann, Bovans and Hy-line. In majority of the farms, multi-aged birds (chicks, growers and multi-aged laying hens) were maintained in the same premises and were reared in cage system or raised cages. Birds were reared under standard managemental conditions recommended by the breeding companies

Methodology

In dead birds, a detailed gross examination was carried out and samples were collected from carcasses which had lesions suggestive of ILT. In such cases, representative tissue samples from trachea, conjunctiva, respiratory sinus and trigeminal ganglia were collected, fixed in 10% neutral buffered formalin (NBF), processed by routine paraffin-embedding technique and 4-5 µm thick sections were prepared. Sections were stained by Haematoxylin and Eosin (H&E) stains for detailed histopathological examinations and in selected cases, Phloxine-Tartrazine staining technique was carried out for inclusion bodies as detailed by Luna [8].

Results and Discussion

Infectious laryngotracheitis (ILT) is a viral disease of the respiratory tract of chickens that has a worldwide distribution and responsible for severe economic losses. The disease occurs in areas where there is intensive poultry production with multi-age egg laying chicken. The disease was first described during 1925 in the USA [9] and subsequently reported in many countries in which it remains a serious problem mainly in areas of intensive production with great concentration of poultry farms rearing birds with multiple age groups [5, 10]. Namakkal (Tamil Nadu, India), the egg town of Tamil Nadu with an area of 200 sq.km concentrating around 550 poultry farms and housing about 50 million layers, undoubtedly satisfied the necessary criteria for the prevalence of ILT. Hence the present study was undertaken at Namakkal, Tamil Nadu and Puducherry, India.

In India, ILT was first reported in Mathura, Uttar Pradesh by Singh *et al.* [11]. Subsequently, it was reported in Andhra Pradesh [12] and Madhya Pradesh [13]. There are no published data on ILT until it was reported in the Namakkal district of Tamil Nadu, India during the year 2012 [14]. Subsequently, many authors have reported the prevalence of ILT in Namakkal, India [15-17] including the present study. This clearly indicates the re-emergence of ILTV infection in India.

During the study period, a single flock of 100 Giriraja birds at Puducherry, India that had gross lesions suggestive of ILT were however found to be negative for ILTV by molecular technique. Further survey studies in this region during reported outbreaks are required to confirm the occurrence disease.

Out of 62 commercial layer farms investigated in the present study, 40 (6.5%) were identified as positive for ILT. In a similar study carried out in the same region for a period of one year, Rajan [18] recorded the occurrence of ILT as 70%. On the contrary, a lower prevalence of 17.33% in Bangladesh [19] and 31.5% in Uruguay [20] has been reported earlier. The variation in the prevalence of ILT in different regions could be attributed to the system of rearing, stocking density and the use of live vaccines.

The occurrence of ILT varied from 4 to 70 weeks of age. Birds between the age group of 10 to 20 weeks were more affected followed by 4 to 10 weeks. The mortality rate ranged from 1% to 17%. In layers, the mortality varied from 1% to 6% with production loss of 12%. The disease was prevalent throughout the year and the course of the disease extended for 30 days. According to Hinshaw *et al.* [21] and Seddon and Hart [22], the epizootic form of the ILT spreads rapidly and can cause high morbidity (90-100%) and mortality (5%-70%) with an average mortality of 10-20%. In a study conducted in Philippines, Saepulloh and Rovira [23] reported that the duration of the illness varied from 2 to 3 weeks with mortality rate of 2 to 22% and production loss of 2 to 15%. A lower mortality rate ranging 1 to 6% was recorded in Brazil [24]. According to Sivaseelan *et al.* [16], the mortality rate progressively increased daily and within 3 to 5 days about 30 to 40 per cent of the flock died. Gowthaman *et al.* [17] (2014) reported that the disease outbreaks were reported around the year and course of disease extended up to 45 days. Kaboudi *et al.* [25] conducted a study involving 48 commercial poultry flocks with history of acute respiratory disease in Tunisia. The age of the flocks under investigation ranged from 3 to 80 weeks. Mortality rate in affected flocks ranged from 6% to 30% and the egg production reduced to 5-20% in layer flocks. The variation in the mortality pattern could be due to intercurrent infections.

In this current study, the affected chicks and grower showed varied clinical signs including dullness, reduced feed and water intake, swelling of the infra-orbital sinuses, closed eyes and respiratory distress. Depending on the severity of the disease, the degree of respiratory signs ranged from mild respiratory distress to difficulty in breathing, gasping, pump

handle-type of respiration (Fig. 1) and sharp laryngeal sounds. Severely affected birds showed expectoration of blood-stained mucus. The clinical signs observed in the present study concurred with the observations of earlier workers [26, 16, 17, 24]. In addition, Beach [27] recorded haemorrhagic conjunctivitis in affected birds.

The gross lesions were noticed mainly in the upper respiratory tract, particularly in trachea. In mild cases, the gross changes in the trachea were congestion and mucoid tracheitis (Fig. 2). In severe cases, hemorrhages or diphtheritic changes, or combinations of these were noticed in the trachea. In such cases, the trachea contained blood clots or yellowish exudate partially/completely occluding the lumen (Fig. 3 & 4). These gross findings concurred with the observation of early workers [28, 29, 16, 17, 24]. The accumulation of mucus and blood varied during the course of the infection and also depend on the strain of the virus. Small amounts of mucus with slight hemorrhages are seen in the early phase of infection and progress to yellow-cheese like mucous plugs in the late stages. The mucus plugs may block the airway and can lead to death by asphyxiation [30].

Fig 1: ILT affected bird; pump hand like respiration with wide open beak.

Fig 2: Trachea: Congestion and mucoid tracheitis.

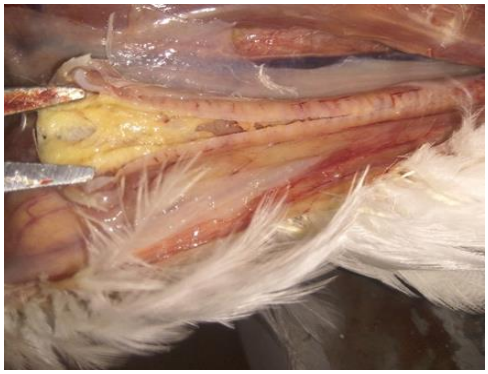

Fig 3 **Fig 4**

Fig 3-4: Trachea: hemorrhages and yellowish exudate completely occluding the lumen.

Histopathologically, trachea showed congestion of mucosal and sub mucosal blood vessels, necrosis (Fig. 5), disruption of the surface epithelial cells, loss of cilia, and infiltration by heterophils, lymphocytes, histiocytes, and plasma cells. The desquamated or the hyperplastic epithelial cells resulted in the formation of syncytia with the presence of eosinophilic intranuclear inclusion bodies (Fig. 6). These findings concur with the observations of earlier workers [28, 31, 24, 16, 17, 25]. The mechanism by which the virus induces the formation of syncytia has not yet been elucidated. Possibly, fusion may be guided by the GaHV-1 virus through the interaction of envelope glycoproteins with cell membrane receptors, similarly to the infection by measles virus, thereby ensuring their replication and spread [32].

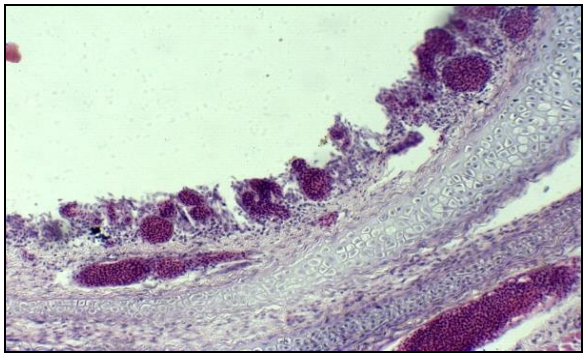

Fig 5: Trachea from ILT affected layer; Congestion of mucosal and sub mucosal blood vessels, H&E stain ×200.

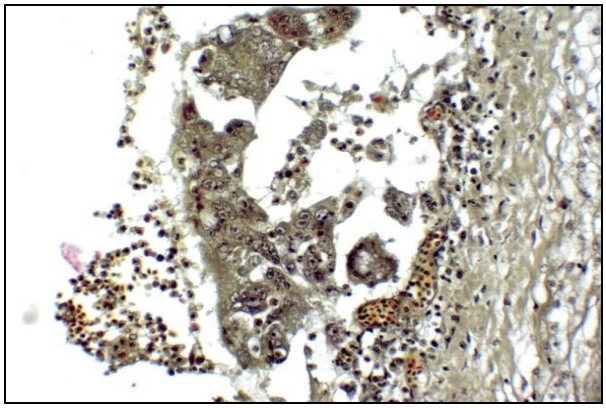

Fig 6: Syncytia with the presence of eosinophilic intranuclear inclusion bodies. Phloxine-Tartrazine stain ×400

Histopathological examination of the trachea for typical intranuclear inclusions is considered characteristic for ILT. In the present study, difficulties were experienced to appreciate the inclusion bodies under routine H&E technique, but the same samples showed inclusion bodies when subjected for Phloxine-Tartrazine staining technique. From this, it is inferable that Phloxine-Tartrazine staining technique could yield better demonstration of inclusion bodies of ILT. These features are similar to those described by Sivaseelan *et al* [16]. According to Hayashi *et al.* [33] and Bagust *et al.* [4], the probability of finding typical inclusion bodies is decreased dramatically when histopathology is performed after eight to 10 days (sub-acute to chronic stage) of infection, due to desquamation of the epithelial cells.

The other changes noticed were cystic dilation of sub mucosal glands and focal or multifocal area of lymphoid aggregates/hyperplasia in the lamina propria of the trachea (Fig. 7). Similar findings have also been reported by earlier workers [34, 24].

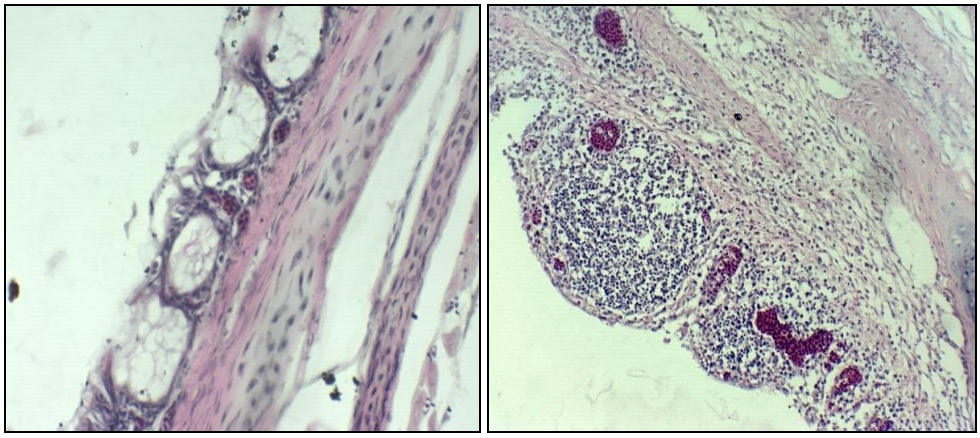

Fig 7: Cystic dilation of sub mucosal glands and area of lymphoid aggregates/hyperplasia in the lamina propria of the trachea. H&E stain×200

In ILTV infection, virus replication is not restricted to the trachea. Both vaccine and field viruses commonly replicate in other tissues like conjunctiva and the respiratory sinuses. In certain cases viral replication has been detected in the air sacs and lungs [35, 36, 26]. However, in present study the conjunctiva did not present any ILT characteristic lesions excepting congestion and infiltration of inflammatory cells highlighting ILTV is highly cytolytic for the trachea as reported by Hitchner *et al.* [37]; Robertson and Egerton [38].

Conclusion

The results of this study demonstrate that ILTV is circulating in laying flocks reared in Namakkal, Tamil Nadu. For this reason, it is important that good management and bio-security practices are developed, improved, and implemented for its prevention and control.

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