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Clinico-epidemiological pattern and economical implication of avian infectious laryngotracheitis in commercial chicken in Tamil Nadu, India

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Abstract
Respiratory disease complex is considered the most serious disease affecting poultry and causes heavy economic losses in the poultry industry worldwide. Among the economically important diseases, Infectious laryngotracheitis (ILT) is a highly contagious upper respiratory tract disease of chicken and has been regarded as a major concern for poultry health and welfare. Keeping in view, the data pertaining to the incidence of ILT were recorded in in various commercial farms in Tamil Nadu. Among 250 birds screened, the incidence of ILT was 34.20%. The highest incidence was observed in 45 layer birds (58.00%) followed by 39 indigenous birds (15.60%), 38 broiler birds (15.20%) and 28 broiler breeders (11.20%). The age-wise highest incidence was recorded in 21-40 weeks old birds (N=14;5.60%). The incidence of ILT was observed throughout the year and the highest incidence was recorded during the months of December (16.00%) and January (15.60%) and thus the highest incidence was recorded in winter (41.20%). The morbidity rate was highest in layer birds (64.67%), followed by growers (26.34%) and brooder chicks (14.53%). The highest mortality rate was recorded in layers (25.45%) followed by growers (14.28%) and brooder chicks (11.39%). The highest reduction in egg production was recorded in the 41-50 weeks old birds compared to the other age groups. Hence, these data served to provide clinico-epidemiological pattern and its economic significance in egg production loss during the study period and to improve the husbandry management and necessary control measures to prevent further outbreak of ILT.

Keywords: Avian**,** infectious laryngotracheitis, TK gene, PCR, age, season, mortality, egg production, incidence

1. Introduction
Poultry farming is one of the rapidly developing sectors, which plays an important role in the global food security. The consequence of globalization, climate change and rapidly expanding poultry population results in the emergence of several diseases. The intensive rearing methods of poultry are responsible for the marked increase in avian respiratory diseases worldwide[1].Various pathogens including Infectious laryngotracheitis virus (ILTV), Infectious bronchitis virus (IBV), Avian pneumovirus (APV), Avian influenza virus (AIV) and Newcastle disease virus (NDV), *Avibacterium paragallinarum*, *Ornithobacterium rhinotracheale* (ORT), *Mycoplasma synoviae* (MS), *Mycoplasma gallisepticum* (MG) and Avian pathogenic Escherichia coli (APEC) are involved in causing respiratory diseases in poultry [3,4,5]. Among the viral respiratory diseases, Infectious laryngotracheitis is considered as the most economically important disease in commercial chicken due to their high morbidity and mortality rate and impact on egg production loss [6, 7, 8, 9]. It is one among the emerging and re-emerging disease listed by world organization of animal health, Office International des Epizootics [10, 11].

In general, periodic occurrence of more severe form of the disease outbreaks of ILT continues to occur, whenever ILTV strains move from persistently infected flocks into non-vaccinated chickens. Moreover, ILT disease status is uncertain due to the irregular or emergency-only vaccination for ILT in some countries [8]. So a keen surveillance study is highly sought. The disease was first reported in India in 1964 and subsequently by many earlier reports [12]. Presently, the disease status has gained momentum as evidenced by the recent reports [13, 14,15, 16] especially in Tamil Nadu. Although the disease affects all ages of bird, the most characteristic signs are observed in adult birds characterized by cough, rales, bloody mucus excretion, marked dyspnoea and decrease in egg production [17-20].

This disease causes production losses due to increased morbidity, moderate mortality, decreased weight gain, reduced egg production and expenses spent on vaccination, biosecurity measures and therapy to counteract secondary infection by other avian pathogens [17, 21, 22, 23].

Keeping in view the high morbidity and mortality rate due to recent cases of ILT in Tamil Nadu and nearby states [14, 15, 16], the present study contemplates to investigate and confirm the incidences and prevalence of suspected field cases of ILT in chicken flocks from Tamil Nadu by molecular detection and characterization techniques.

2. Materials and Methods

2.1. Epidemiological sample collection

The present study was carried out for two years between October, 2019 to September, 2021 in the Department of Veterinary Pathology, Madras Veterinary College, Chennai. The data regarding breed and strain of chicken, flock strength, age, method of rearing, previous outbreak of disease if any, production performance, clinical signs manifested and rate of morbidity and mortality were collected from various farms having the history of respiratory signs in and around Namakkal, Tiruchirappalli, Pudukkottai and Thanjavur districts of Tamil Nadu. Necropsy was carried out on both ailing birds and fresh carcasses brought to the Poultry Disease Diagnosis and Surveillance Laboratory (PDDSL), Namakkal, Regional Research and Educational Centre (RREC), Pudukkottai and also on other birds during farm visits in nearby districts having the history and clinical signs of respiratory illness. Samples of oro-pharyngeal swabs and organs such as paranasal sinuses, larynx, trachea, lungs, air sacs, kidneys, ureter, oviduct, spleen, harderian gland, caecal tonsil and bursa of Fabricius were collected from the sick birds and dead birds showing lesions for confirmation of Infectious Laryngotracheitis virus using TK gene based Polymerase Chain Reaction as per OIE manuals [24].

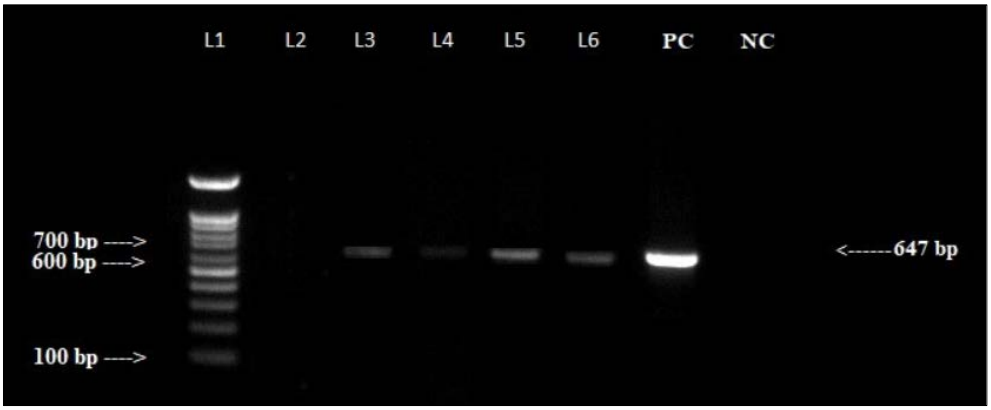
2.2. TK gene specific Polymerase Chain Reaction detection

Molecular detection of viral genome was carried out in clinical specimens by conventional PCR using ILTV specific primer-TK gene in a PCR thermal cycler (Eppendorf, USA) as per standard protocol from OIE manuals [24].

3. Results and Discussion

3.1. Pathogen detection by TK gene-specific PCR

The confirmative detection of ILTV was done by subjecting the swabs and tissue samples by conventional PCR. Samples positive for ILT revealed amplicon size of 647 bp for TK gene (Figure 1).



1.5% Agarose gel electrophoresis showing the 647 bp PCR product of TK gene of ILTV
Lane 1- 100 bp ladder, Lane 2 - ILT negative, Lane 3-6 - ILT positive, Lane 7-Positive control, Lane 8- Negative control

Fig 1: Amplification of ILT virus by TK gene specific PCR

3.2 Overall Incidence of ILT

A total of 720 birds were screened in which 250 birds were found positive for ILT during the study period. The incidence of ILT among respiratory diseases was 34.20%. Among the ILT affected flocks, age-wise, month-wise and season wise incidences were recorded. The findings of the present study are in agreement with the earlier work [14] who investigated in 32 commercial flocks from seven Indian states and reported that 31.25% (10 flocks) were found positive for ILTV infection by PCR. The report elucidated that virus persistence in farm environments leads to spread of disease. Similarly, Mishra *et al*. (2020) [16] reported that a total of 22 out of 26 flocks positive for ILTV infection and our findings are in accordance with their report in the same region indicating the persistence of ILTV infection in densely populated poultry region of Tamil Nadu. [27, 28, 29, 30].

3.3 Age wise incidence

In the present study, among 250 positive cases of ILT, the highest incidence was observed in 45 commercial layer birds (58.00%) followed by 39 indigenous birds (15.60%), 38 broiler birds (15.20%) and in 28 broiler breeders (11.20%), respectively (Figure 2). Among the broiler birds, the highest incidence was recorded in the age group of 0-3 weeks (8.40%). Among the broiler breeders, the highest incidence of ILT was recorded in the age group of 0-20 weeks (6.40%). In the indigenous type of birds, 21-40 weeks old birds showed the highest positivity (5.60%) for ILT. Similarly, among the layer birds, 0-20 weeks aged birds (27.20%) showed the highest incidence of ILT.

In our study, the ILT infection was recorded in broilers, indigenous birds, broiler breeders and layers and similar occurrence of ILT infection among chicken was reported in both broilers and layers (Jordan, 1966;Gowthaman *et al*., 2014) [31, 14, 32]. Reports from various countries also pointed outbreaks in broiler farms which altered the concept that ILT is a disease primarily concerning adult laying birds [33, 34]. These outbreaks had been provoked by the proximity of broiler farms to vaccinated layer hens and backyard flocks, the intensive way of poultry farming with shorter cycles of

production, and biosecurity gaps (Gowthaman *et al*.,2020) [30].

3.4 Month wise incidence

In the present study, the highest incidence of ILT recorded was in the month of December (16.00%), followed by June (15.60%), November (15.20%), April (10.00%), May (9.60%), January (6.00%), July (5.60%), September (5.20%), March (4.28%), August (4.40%), February (4.00%) and October (3.60%) respectively (Figure 2). Though the monthly incidence varied, ILT was found to be prevalent almost throughout the year but was mostly prevalent in December and June.

These findings were supported by the reports from the same geographical location [14, 16]. The findings indicated that the environmental factor such as extreme hot and cold climates might trigger the disease frequency due to stress and immunosuppression as reported in other viral disease outbreaks reviewed that most ILT caused significant respiratory problem in cooler seasons of the year [27, 39]. Similar type of study was done in which reported highest 24.4% seroprevalence of ILTV in the winter season [40]. Poor biosecurity measures and birds rearing in deep litter rearing were most susceptible for ILTV infection [5]. The environmental factors such as change in climatic conditions, relative humidity, poor ventilation and managemental related problems probably influenced the outcome of the present study.

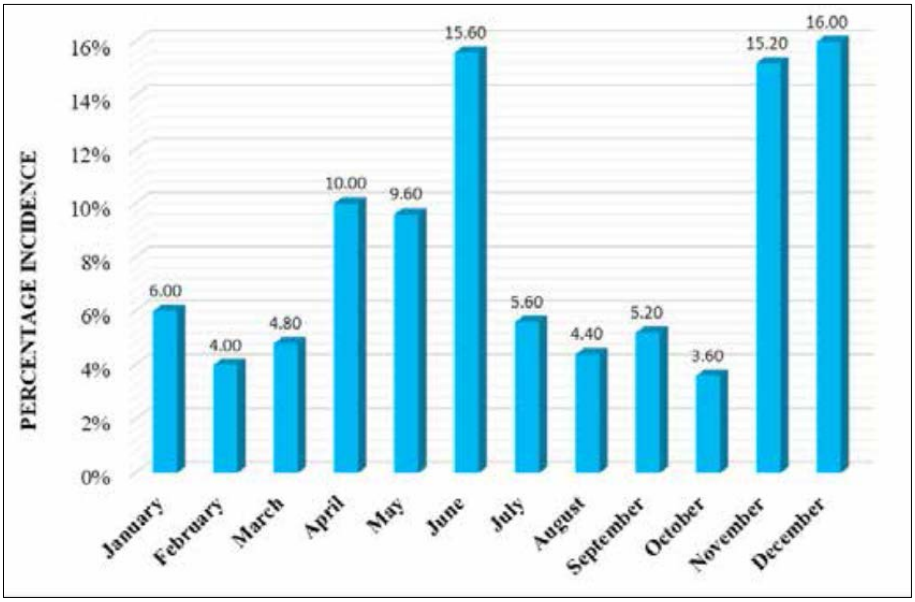


Fig 2: Month-wise incidence of ILT

3.5 Season wise incidence of ILT

Season wise incidence of ILT in the present study showed the highest incidence in winter season (41.20%) followed by summer (40.00%) and monsoon season (18.80%), respectively (Figure. 3). The disease has been reported to occur throughout the year *viz*., winter, summer and even in rainy season [14, 41]. However, higher seroprevalence of the disease in winter (24%) compared to rainy (16%) and summer (12%) seasons were reported [40]. The findings supported the current investigation of season wise incidence.

Another study reported that the disease was prevalent all-round the year especially, in summer and winter followed by rainy season [16]. An extreme cold climate in winter and a hot and humid climatic condition in summer prevailing in the study area might be the contributing stress factors for the affected flocks. Hence, seasonal variation, weather, and past history of infections could be associated with compromised immune response in the affected flocks with viral disease [30].

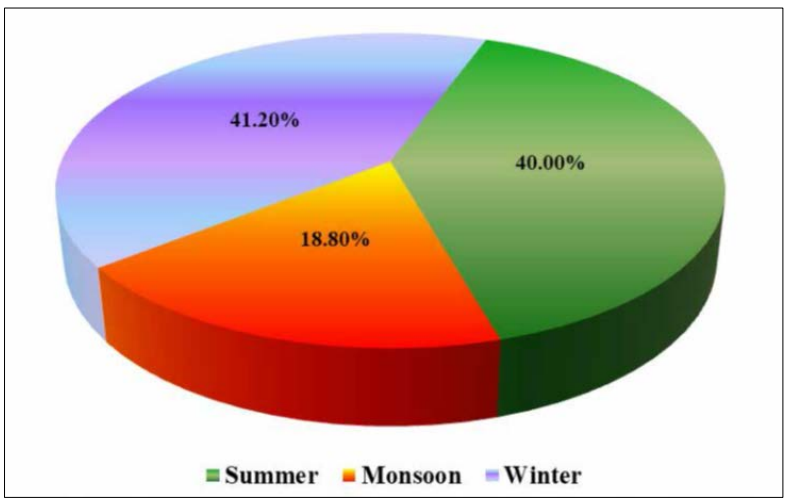


Fig 3: Season wise incidence of ILT

3.6 Morbidity and Mortality pattern

The morbidity and mortality pattern in ILT affected chicken in the study showed the highest morbidity rate in layer birds (64.67%) followed by growers (26.34%) and brooder chicks (14.53%), respectively. The mortality rate was higher in layers (25.45%) followed by growers (14.28%) and brooder chicks (11.39%), respectively (Figure. 4).

ILTV usually causes a reduction in egg production and variable mortality ranging from 0 to 70% [42]. The morbidity is high with levels from 50 to 100%, with the mortality levels varying *et al.* from 0.1% in mild cases to 70% in severe outbreaks [40] depending on the virus strain, the viral load, the concurrent diseases, and the health status of the flock, among other factors.

It was reported that mortality might go up as high as 50% or more in peracute cases and between 10-30% in subacute cases [43]. The mortality rate in ILT affected flocks of present study ranged from 11.39% to 25.45%, indicating that the subacute form of ILT was dominant in the present study.

The variable nature of mortality and morbidity among brooder, grower and layers could be mainly due to the variations in stress factors, host-associated (age, immune status), virus-associated (strain, pathogenicity, virulence and tissue tropism), or environmental (cold and heat stresses, dust, and presence of ammonia) factors those prevail in the area of disease outbreaks [30]. The highest incidence of mortality and morbidity rate in layer flocks was observed in the present study which might be due to reactivation of latent infection and subsequent shedding of virus periodically, particularly during times of stress such as start of lay and peak egg production [46].

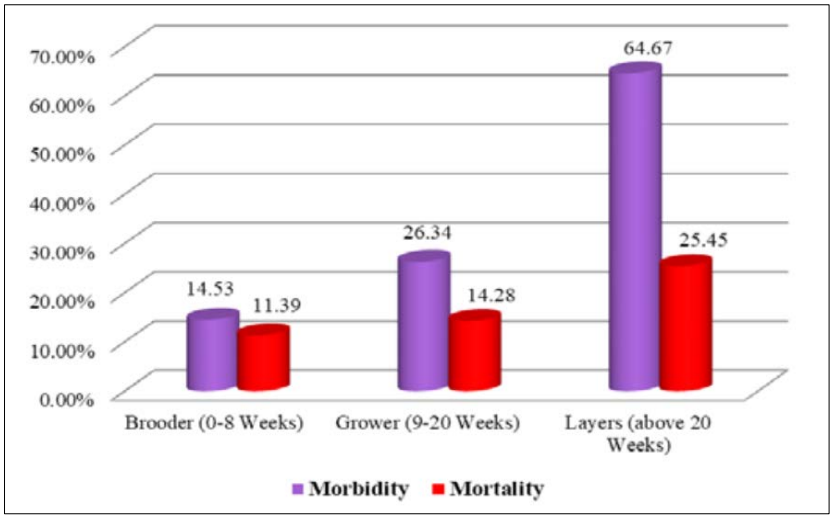


Fig 5: Morbidity and mortality pattern in ILT affected chickens

3.7 Egg Production in ILT affected birds

The egg production pattern in ILT affected birds showed a highly significant decrease in egg production when compared to healthy flocks in the age groups from 18-20 to 71- 80 weeks (Figure 6). Among the different age groups, the highest reduction in egg production was recorded in the 41-50 weeks old birds compared to other age groups.

Several reports have described that ILT infection causes varying level of egg production in layer flocks, with the complete cessation of egg production in some flocks [33, 13, 14, 47]. It is inferred that the ILT infection impairs the general physiological health status of the infected bird leading to fall in egg production as a secondary effect to the infection [48].

It was stated that ILTV undergoes systemic replication and these findings support the speculations that the infection of reproductive tract and replication of ILT virus in oviduct might be associated with lesions of the oviduct, leading to decreased egg production during ILT outbreaks [49,50, 51,52].

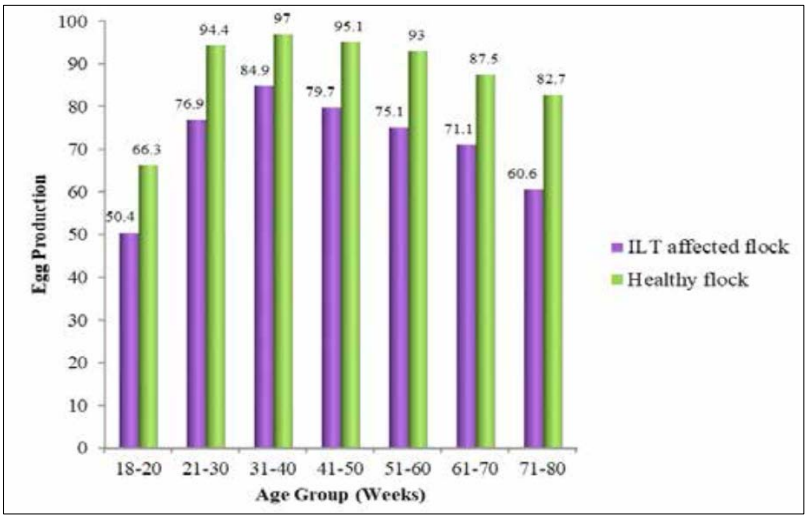


Fig 6: Egg production pattern in ILT

4. Conclusion

The Incidence of ILT recorded in this present study was 34.72% among the 720 birds. Prevalence of ILT showed the incidence in all type of chicken and highest incidence of ILT was recorded in layer (58.00%). The age wise incidence of ILT was observed in all age groups and highest incidence was recorded in 21-40 weeks old birds (5.60%). The incidence of ILT was observed throughout the year and highest incidence was recorded during the months of December (16.00%) and January (15.60%) and season wise incidence produced highest incidence in winter (41.20%). The morbidity rate in ILT ranged from 14.53 to 64.67% and mortality rate was11.39% to 25.45% and highest rate of egg production loss was observed in birds of age group 41-50 weeks. This data would help to take necessary control and preventive measures for ILT in commercial and backyard poultry farms.

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