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## Original Article

# Serological Survey of Infectious Laryngotracheitis in Broiler Flocks, Iran, 2018

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## Abstract

**Background and Aims:** Infectious Laryngotrachitis (ILT) is an acute respiratory disease with high morbidity and low mortality in poultry. ILT is caused by Gallid herpesvirus 1 (GaHV-1), a member of the Iltovirus genus and family Herpesviridae. It causes in notable economic losses due to decreasing the growth rates, egg production, and increasing the mortality in commercial poultry, especially layer flocks, and usually, outbreaks are more severe in older birds than in younger flocks. However, Infectious Laryngotrachitis has been reported in broiler as well. Multicausal respiratory diseases are prevalent diseases in Iranian broiler flocks and caused a high rate of mortality and considerable economic losses. Field and vaccine strains of Infectious Laryngotrachitis virus are circulating in layer flocks in some geographical locations.

**Materials and Methods:** To detect seroprevalence of the Infectious Laryngotrachitis virus in broiler flocks, a total of 180 sera samples were collected in slaughterhouses from 15 broiler flocks (12 sera from each flock) of four provinces in Iran containing Golestan, West Azerbaijan, Qazvin and Tehran during autumn 2018. Infectious Laryngotrachitis virus antibodies were determined using a commercial ELISA test kit.

**Results:** Based on the results, the seroprevalence of the Infectious Laryngotrachitis virus was found to be 13 % of flocks. Also, our finding showed that 33% and 25% of flocks were seropositive to ILTV collected from Tehran and West Azerbaijan respectively.

**Conclusion:** The results revealed that Infectious Laryngotrachitis viruses are circulating in broiler flocks in different parts of Iran. This is the first report of ILT Seroprevalence in broiler flocks in Iran and in future, the molecular studies on ILT would be nessaccery in respiratory disease syndrome.

**Keywords:** Seroprevalence; Infectious Laryngotracheitis; Broiler; Iran; ELISA

## Introduction

Infectious Laryngotracheitis (ILT) is an important, highly contagious and acute respiratory tract infection of chicken described in 1925 for the first time [1]. The

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causative agent of ILT is *Gallid herpesvirus 1* (GaHV-1), a member of the *Iltovirus* genus and family *Herpesviridae* within the order *Herpesvirales* [2].

There are two types of disease including; Severe epizootic forms of infection which are characterized by respiratory distress such as gasping and expectoration of bloody mucus, high morbidity, and moderate-to-high mortality through occlusion of trachea, Milder enzootic forms of infection which occurr often in developed poultry industries and appear as mucoid tracheitis, sinusitis, conjunctivitis, general unthriftiness, and low mortality.

The disease results in significant economic losses by adversely affecting growth rates, egg production, increasing mortality, and loss of export markets [3, 4]. Preventive strategies are usually based on vaccination by using two types of ILTV live attenuated vaccines, the chicken embryo origin (CEO) which is attenuated by sequential passages in embryo-nated eggs (Using in Iranian poultry industry); and the tissue-culture origin (TCO) which is generated by sequential passages in tissue culture [5-7].

According to studies, live attenuated vaccines especially the CEO, can spread from bird to bird in close contact and revert to virulence after some passages [8]. There are limited studies about ILT in Iran, which almost conducted in commercial layer flocks [9-11].

Purposeful vaccination strategy against ILT is available in Iran using live attenuated vaccines in some provinces of the country where the ILT is prevalent in breeder and layer flocks. In recent years, several outbreaks of the disease in broiler flocks have been reported by the Veterinary Organization of the country. Since there is no vaccination program against ILT in Iranian broiler flocks, this study was carried out to determine the serological status of ILT in these types of poultry in some high-risk provinces of Iran.

## Methods

**Study population.** This cross-sectional study was conducted during Sep-Dec 2018 in Qazvin, Tehran, Golestan, and West Azerbaijan provinces where there is a high density of poultry population and also some reports of ILT outbreak in breeder and layer flocks in these regions.

To determine the prevalence of disease 15 flocks from Golestan (4 farms), West Azerbaijan (4 farms), Qazvin (4 farms) and Tehran (3 farms) were selected, and 12 birds were bled per each farm based on 20% prevalence, 95% confidence level to identify at least one positive serum of bird [12].

The chickens were 42-days-old and were reared in farms containing 20000-40000 chickens. The flocks had not received any ILT vaccines during the rearing period.

**Enzyme-linked immunosorbent assay (ELISA).** Collected sera were assayed for ILTV antibodies. Serum antibody titers to ILTV were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit **(**Synbiotics Corporation, ProFLOCK_, San Diego, CA**)** following the instructions of the manufacturers.

The absorbance at 450 nm was read using a SPECTRAmax_ELISA reader (Molecular Devices, Sopachem, Brussels, Belgium). The presence of antibody in blood serum samples were determined by calculating sample to positive control ratio. The result was accepted as positive for ILT when the sample was having the S/P ratio of greater than 0.50. If the S/P ratio of the sample was less than 0.50, it was considered negative.

**Statistical analysis.** All analyses were conducted using Microsoft Excel and the GraphPad Prism software, V.6.0.1.

## Result

In this study, 13% (2 of 15) flocks were found to be positive for the ILT virus antibody, and belonged to Tehran and West Azerbaijan provinces. Among samples, the sera collected from provinces, including Tehran (3351.833± 1603.204) and West Azerbaijan (2628.6667± 2954.6693), exhibited positive antibody titer. Antibody titers of ELISA tests are shown in figure 1. Suspected farms in Tehran had 33% positive sera samples.

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However, 25% of samples obtained from such farms in West Azerbaijan province were found to be positive for ILTV antibodies (Table 1).

## Discussion

ILT outbreaks several have been reported in layer and breeder flocks in Iran since 1994 [9, 11]. Aghakhan et al. reported of ILT outbreak in commercial poultry, particularly layer and breeder flocks [9]. The other study was conducted on 17 commercial layer flocks from different provinces of Iran during 2003, and 84.3 % of flocks were found to be ILT positive in the PCR technique [11]. Concurrently Ashrafi et al. detected ILT occurrence for the first time in a layer flock of East- Azerbaijan province by histopathologic and serological tests [10].

In the present study, serum samples were collected from 15 broiler farms. One farm in Tehran province and another one in West Azerbaijan province were found to be seropositive against ILT. In both provinces, ILT vaccination is implemented in layer and breeder flocks, and the vaccinal virus maybe is spread to broilers. Furthermore, due to the absence of related clinical signs in these suspected broiler flocks, the circulation of the vaccinal virus is more probable. In Qazvin province, there is a high density of breeder and layer flocks, although the number of broiler farms is low, and this can be one of the probable reasons for seronegative broiler farms against ILT in this province. All samples in Golestan province were negative. In this province, the density of broiler flocks is higher. However, there are fewer breeders, and layer flocks in this region and the vaccination programs are not implemented against ILT in Golestan. Therefore, using live attenuated vaccines against ILT in two seropositive provinces can be the main reason for ILT

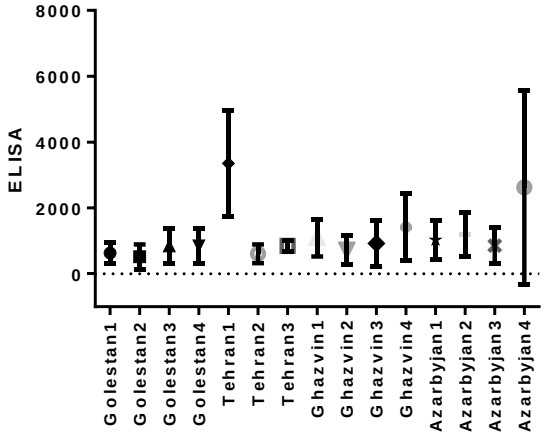


**Fig. 1.** Range of anti-ILTV antibody titers in sera collected from different provinces of Iran.

detection, but performing the molecular tests and sequencing of circulating viruses in the country is necessary to confirm the results.

Generally, vaccination strategies with live attenuated vaccines against ILT have proven to be an effective method for developing resistance in susceptible poultry. However, this is only recommended for use in geographic areas where the disease is enzootic because of living attenuated vaccine strains possible ability to establishing lifelong latent infections and transferring to naïve sensitive chickens by horizontal routes [13]. Iran Veterinary Organization, as a regulatory agency, determines justifiability of provinces to use of approved Chicken Embryo Origin (CEO) type of ILT vaccines due to the issue above. Based on such circulars, ILT vaccination in Tehran, West Azerbaijan, and Qazvin provinces are allowed to apply in layer and breeder flocks while it is illegal in Golestan province.

ILT has been reported from all over the world, including Brazil, China, North and South of America, Europe, Australia, Africa, southwest Asia, New Zealand and Turkey [14-17]. Reports of ILT outbreak in broilers are limited because of its tendency to affect the layer

**Table 1:** Rate of positive flocks against ILT in broiler flocks in Iran (Four Provinces).

| Provinces | Positive | Negative |
|---|---|---|
| Tehran | 33% (1/3) | 67% |
| West Azerbaijan | 25%(1/4) | 75% |
| Golestan | 0% | 100% |
| Qazvin | 0% | 100% |

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flocks at older ages. During 2001 mild ILT outbreak in three broiler flocks with no increased mortality and the minimal serological response has been reported from the southeastern of the United States. The broilers had mild respiratory signs and were not vaccinated against ILT; however, in our study, broilers were clinically healthy and had no respiratory signs [17]. Chacon et al. conducted a molecular study by nested PCR assay on trachea and lung samples collected from 51 commercial layer farms from Brazil in 2007 and found 45% ILTV positive among samples [15]. Similar to our seroprevalence study, a serological survey was done by using the ELISA test on 300 blood samples from Bangladeshi non-vaccinated commercial layer flocks in 2014 and 17.3% ILTV seropositive samples was reported [18].

Additionally, the seroprevalence of ILT in layer flocks among the four states of India was found to be 26.77% according to ELISA test results, and the authors indicated that ILT is more prevalent in winter and smaller age (10-35 week) [19]. Recently in 2018, another serological assay has been conducted on non-vaccinated commercial layer flocks in Turkey by using the ELISA test, which showed that 16 of 25 farms (64%) were ILTV seropositive [14]. Interestingly, almost of ILTV positive flocks foresaid, like the current study, were not vaccinated against ILT, and the origin of infection was not determined [14, 15, 17, 18].

In vaccinated layer flocks, pullets vaccinated with CEO ILT vaccines and transferred to a breeding farm or laying house with probable shedding the virus under the stress of transportation. If the pullet transporter has contacted with a naïve broiler flock promptly after having contact with the ILT-shedding pullets, or if the same vehicles have used to moving broiler flocks, it could be resulting virus transmission by direct contact within the broiler flock, and maybe start a broiler ILT outbreak [20-22].

Backyard flocks are considered as an important source of the ILTV to the other birds because they can easily be infected due to lack of biosecurity procedures and survived birds most likely will become a reservoir of the virus. A risk analysis study concludes that broiler flocks have been affected with ILTV, 36 times more likely to be located within 1.6 Km of a backyard flock than unaffected poultry flocks [23].

Recently ILT has been reported from neighboring countries such as India, Turkey, and another region from Southeast Asia [14, 18, 19]. Infected bird exchanges in borderline centers and possible transmission of the disease by migratory birds can be considered as reasons for the ILT outbreak in Iran.

Besides, vaccination against ILT does not carry out in broiler flocks in Iran. Our finding showed that ILT is circulating in broiler flocks in different parts of Iran.

There are several probabilities for the presence of seropositive broiler flocks in the country, including vaccinated layer flocks, infected carrier back-yard poultry, and poor biosecurity measures.

There is a serious need to conduct more studies around ILT and its prevalence in both layer and broiler flocks in Iran and determination of the origin of the disease by characterization of causative agents. Also, hardline control measurements will be required to decrease the ILT occurrence and, subsequently, its adverse effects in poultry production.

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