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Isolation of infectious laryngotracheitis virus in broiler chicken in Iran: First report

Jamshid Razmyar[1], Sara Shokrpoor[2]*, Abbas Barin[3], Jamshid Gheshlaghi[4], Peyman Nakhaee[5], Moein Khodayari[1], Seyed Mostafa Peighambari[1]

[1] Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [2] Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [3] Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [4] Private Poultry Clinic and Diagnostic Laboratory, Hamedan, Iran; [5] Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

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Abstract

In February 2019, a severe respiratory distress with co-infection of infectious laryngotracheitis (ILT) and Newcastle disease accompanied with *Salmonella Enteritidis* occurred in a broiler flock in the western region of Iran. Clinical signs included paralysis, torticollis, nasal discharge, conjunctivitis, gasping and respiratory rale with high mortality. At necropsy, caseous diphtheritic membrane adherent to the larynx and trachea was observed. Microscopically, syncytial cells formation with dense eosinophilic intranuclear inclusion bodies were main histopathological findings in tracheal tissues. Conventional polymerase chain reaction (PCR) for ICP4 gene amplification as a definitive diagnosis was utilized for the detection of ILT virus nucleic acid in suspected tracheal samples inoculated on to the chorioallantioc membrane of 11-day-old specific pathogen free (SPF) chicken eggs. Tracheal tissues taken from these SPF birds were positive by nested ILT PCR. In conclusion, because of no vaccination policy against ILT in broilers, the most probable scenario is that virus-laden dust or other fomites can be vectors and virus persistence and disease outbreak can be a sequel of wild virus introduction to the farm.

Introduction

Infectious laryngotracheitis (ILT) is an acute and transmissible upper respiratory tract disease occurring mainly in chickens becoming one of the most significant diseases in broilers.[1] In spite of more susceptibility of chickens older than three weeks of age, ILT has been recognized in broiler chickens as young as three weeks of age.[1] The disease is identified by clinical symptoms like conjunctivitis, respiratory distress, gasping, sinusitis, unthriftiness, coughing, neck stretching, asphyxiation and tracheitis.[2] Since there is no treatment for the disease, strategies for controlling ILT are generally based on biosecurity and/or vaccination similar to the most of other poultry diseases.[3] Because clinical signs and even gross lesions of ILT are similar to other respiratory diseases, diagnosis should be based on laboratory diagnostic methods including histopathology, virus isolation, serological tests and DNA based methods like polymerase chain reaction (PCR).[4]

The aim of this report is to describe clinical, pathological, histopathological and molecular aspects of first documented outbreak of ILT occurring concurrently with Newcastle disease (ND) and salmonellosis in a commercial broiler flock located around Sonqor, Kermanshah province, Iran.

Case Description

A broiler flock was composed of 25,000 Ross 308 broiler chickens placed in two separate houses around Sonqor, Kermanshah province, Iran. In February 2019, high mortality was started from one of the broiler chickens hall at 20 days of age with dominant respiratory clinical signs. Clinical signs included paralysis, torticollis, nasal discharge, conjunctivitis, gasping and respiratory rale with high mortality. The administrated vaccines in the farm were as follows: A spray vaccine at 5-day-old (Cevac® Vitabron L; Ceva, Libourne, France), an inactivated ND

***Correspondence:**

Sara Shokrpoor. DVM, PhD
Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
E-mail: shokrpoor@ut.ac.ir

vaccine injected at 8-day-old (Newpasol 101®; Pasouk, Tehran, Iran) and eye drop vaccination at 12-day-old for both infectious bronchitis (NOBILIS® IB MA5; Nobilis Health Corp., Houston, USA) and ND (Avinew®; Boehringer Ingelheim, Ingelheim, Germany), simultaneously. Firstly, according to the related lesions of necropsy findings and also based on presumptive diagnosis of ND, emergency vaccination (Avinew®; Boehringer Ingelheim) via drinking water method was implemented. To prevent secondary bacterial infections, combination of erythromycin (Rooyan Darou, Tehran, Iran) at a dose of 1.00 g per L in 24 hr and fosfomycin (Rooyan Darou) at a dose of 160 mg kg^{-1} in 8 hr of a day was supplemented in drinking water for five days. After five days and more than 4,000 mortalities in house A, the disease started in the second hall. From each broiler hall, 10 broilers were delivered to the Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Total mortality of the farm reached up to 18,000 birds (72.00%) of the total size of the flock. Chickens were euthanized with cervical dislocation according to National Institute of Genetic Engineering and Biotechnology (NIGEB) animal care and use committee (ethical code No. IR.NIGEB.EC.1397.11.30F). Necropsy and sampling for bacteriological and histopathological examinations were done. Samples of trachea were also pooled and submitted for virus isolation and PCR was performed as described previously.[5-7]

Gross lesions were located mainly in the upper respiratory tract (particularly in trachea), proventriculus, cecal tonsils, cecum and small intestine. In six chickens, tracheas were filled with yellowish fibrinous or caseous diphtheritic membranes adherent to the larynx and upper respiratory tracheal mucosa blocking the larynx and cranial part of the trachea (Fig. 1A). In other four chickens, hyperemia in larynx and especially in the cranial and medial parts of tracheal mucosa was seen. There were also severe hemorrhages at proventriculus (Fig. 1B) and cecal tonsils (Fig. 1C) and white cecal cores were obvious in a number of postmortem findings (Fig. 1D). *Samonella enteritidis* was isolated from cecal samples using the World Organization for Animal Health protocol. Histopathological examination of the trachea showed severe hemorrhagic fibrinous tracheitis.

There was a heavy thickening of the mucosa due to numerous polymorphonuclear and cellular debris enmeshed in a framework of fibrin and granular eosin staining debris. In addition, epithelial cells necrosis, hyperplasia and desquamation along with fibrin, cellular debris and heterophils formed a diphtheritic membrane. The capillaries were congested and hemorrhage was also seen (Fig. 2A). The exudate cells were abundant in various stages of degeneration and necrosis. Detached syncytial cells with dense eosinophilic intra-nuclear inclusion bodies were observed in the trachea (Figs. 2B and 2C).

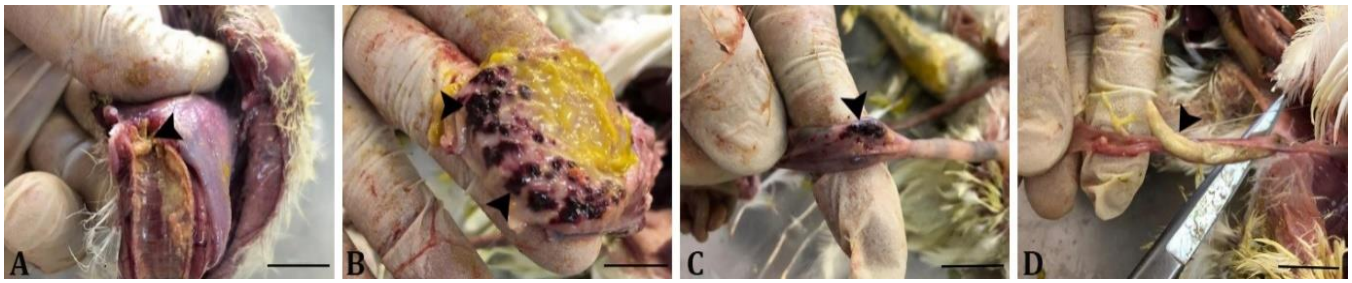

Fig. 1. A) Infectious laryngotracheitis: Clotting blood, fibrinous and caseous pseudomembranous (arrowhead) in trachea. **B)** Newcastle disease: Severe hemorrhage in proventriculus (arrowheads). **C)** Newcastle disease: Typical hemorrhage in cecal tonsils or Peyer's patches of the small intestine (arrowhead). **D)** *Salmonella enteritidis*: Firm and cheesy material or cecal cores (arrowhead) in the cecum, (Scale bars = 1.00 cm).

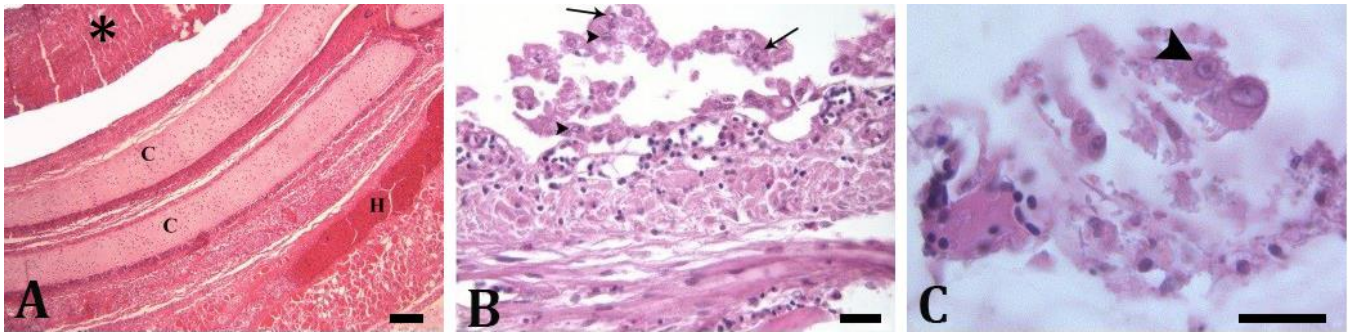

Fig. 2. A) Infectious laryngotracheitis: tracheal lumen is filled with mucohemorrhagic exudate with fibrinonecrotic debris (asterisk). C: Cartilage; H: Hemorrhage. (H&E, Scale bar = 100 µm). **B)** Infectious laryngotracheitis: Detached syncytial cells with eosinophilic intra-nuclear inclusion bodies (arrowheads) and necrotic cells (arrows) are observed in the trachea, (H&E, Scale bar = 20.00 µm). **C)** Infectious laryngotracheitis: High magnification of sloughing tracheal epithelial cell with eosinophilic intra-nuclear inclusion body (arrowhead), (H&E, Scale bar = 20.00 µm).

Both specific genus and species PCR assays for *Salmonella* and ND virus were positive. As shown in Figure 3, the amplified part of the ICP4 gene with specific primers showed expected product size of 635 bp at agar gel electrophoresis proving the presence of viral DNA of ILT in suspected tracheal samples.

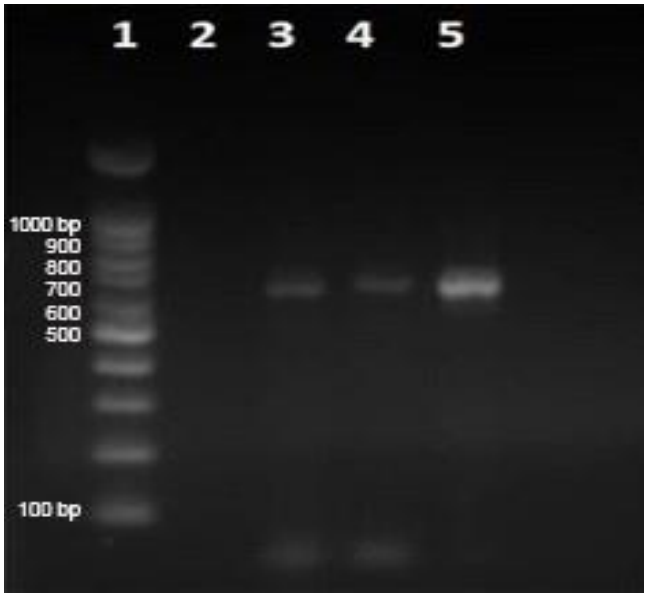

Fig. 3. Polymerase chain reaction results of ICP4 gene amplification. Lane 1: DNA ladder (SINACLON, Tehran, Iran); Lane 2: Negative control; Lane 3: Tracheal tissue sample; Lane 4: Chicken embryo origin vaccine; Lane 5: Chorioallantioc membrane of embryonated eggs.

Discussion

Respiratory complex infections can influence poultry health and productivity leading to economic losses.[8] The ILT is a widespread respiratory disease of chickens. In spite of extensive vaccination with live attenuated vaccines, the disease induces remarkable economic losses in areas with intensive production of broilers. This topic is true for layers but not for broilers in our country and outbreak of ILT infection in Iran broiler production industry could be a serious challenge with consequences. Therefore, because of co-incidence of velogenic ND and ILT as two viral infections with clinical respiratory presentation, this is not possible to relate the respiratory symptoms of chickens to the each of viral agents. Therefore, in this case report we could not accurately determine the form of ILT and its clinical signs in broilers. While, in other reports, the researchers have obviously described the severity of clinical presentation of ILT in broilers.[1,2,9] The results of the culture test from intestinal contents of some birds demonstrated the infection with *S. enteritidis*. The source of infection can be contaminated feed, biological vectors and mechanical carriers such as mice and insects or even farm workers. In this case report, since the exposure routes were copious, the exact route determination was difficult. According to the other reported outbreaks of ILT in broilers, the most common necropsy lesions were hemorrhage and caseous/ fibrinonecrotic membrane in the larynx and trachea.[2,10] The histopathological diagnosis is considered as a valid and relatively rapid method for ILT; but, since the possibility of finding typical lesions is decreased depending on the stage and severity of infection, histopathological analysis must be implemented as soon as possible, during the first three to four days of infection, before any destruction of the respiratory epithelium. Actually, specific lesions could diminish after that time due to epithelial cell desquamation in the upper respiratory tract.[10] In this report, histopathological findings revealed presence of syncytial cells with eosinophilic intra-nuclear inclusion bodies in most of tracheal tissues which are pathognomonic for ILT and these findings are in agreement with other studies;[6] however, some other researchers have reported infrequent intra-nuclear inclusion bodies in different fixed tissues such as eyelid and primary and secondary bronchi due to sampling time.[11,12] In cases where the significant lesions of ILT are missing or complicated with other diseases, ancillary diagnostic tests are highly recommended such as PCR for definitive diagnosis. The PCR is considered more sensitive than virus isolation and conventional PCR was implemented successfully in this study in association with histopathology for definitive diagnosis of ILT in broilers. Also, the primers designed to amplify a product from the ILT virus ICP4 gene demonstrated good sensitivity.[11] There have been various reports and diagnoses of ILT infection in poultry especially broilers by these methods as confirmed techniques[2,6,9-11] being used in this case report as a first outbreak of ILT in broilers in Iran. There are some studies explaining ILT virus as one of the pathogens involved in respiratory complexes, for example, in a study reporting co-infection of fowl pox and ILT[11] or in another study which the investigators have reported the concurrent infection of ILT virus with *Mycoplasma* spp, fowl Pox and *Avibacterium* spp.[13] This is the first report demonstrating the presence of ILT virus in the respiratory complex simultaneously with ND. Because of the quarantine and eradication policies against ILT in Kermanshah province, Iran, no ILT virus vaccine had ever been used in the farm and this was the first outbreak of disease. It should be noted that biosecurity precautions and disinfection processes were not implemented in this farm, so the most probable scenario is that virus-laden dust or other fomites were transported to the farm by visitors, personnel, feed trucks and any factor that can be a vector for virus. Therefore, virus persistence and disease outbreak can be a sequel of wild virus introduction to the farm. In this case, there was also no farm in proximity, especially layer farm, thus it seems that this is not a reason

for occurrence of disease. As mentioned, vaccination and standard biosecurity are two main intervention strategies for ILT. However, there are some reports showing that live vaccines, especially chicken embryo origin ones, can regain their virulence after bird to bird passage.[14]Another disadvantage of live vaccines is latent infection which can cause disease in stressful conditions.[7] Therefore, it seems that vaccination against ILT in broilers could be an appropriate preventive tool in high risk areas of Iran. In general, occurrence of three simultaneous infections including ILT and ND in respiratory tract and salmonellosis in gastrointestinal tract can be a good reason for biosecurity gap in the farm; however, infection exact routes determination can be very difficult.

In conclusion, because of no vaccination policy against ILT in broilers, the most probable scenario is that virus-laden dust or other fomites can be vectors and virus persistence and disease outbreak can be a sequel of wild virus introduction to the farm.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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