Avian Infectious Laryngotracheitis

Zeinab M. S. Amin Girh[1], Nagwa S. Rabie[1] and Mona S. Zaki[2]

[1]Department of Poultry Diseases, National Research Centre, Dokki, Giza, Egypt.
[2]Hydrobiology Department, National Research Centre, Dokki, Giza Egypt.
Dr_mona_zaki@yahoo.co.uk

Abstract: Avian Infectious Laryngotracheitis (AILT) is a respiratory tract disease of great importance because it causes significant economic losses in the poultry industry around the world**.** The target system for Avian Infectious Laryngotracheitis virus (AILTV) infections is the respiratory system, and the main organ in which the virus remains latent is the trigeminal ganglia. However, the virus has demonstrated tropism for other organs besides the respiratory tract. The main transmission routes are ocular and respiratory. Clinical signs usually appear 6-12 days after natural exposure and may be moderate or severe. The causative agent of this disease can be propagated in chorioallantoic membrane (CAM) of developing chicken embryos and replicate in mature chicken kidney cells, as well as in a variety of epithelial chick embryo cells, such as kidneys, liver and lungs. There are several procedures for the diagnosis of ILT such as the observation of clinical signs, the detection of gross and histopathological lesions, and the use of molecular techniques, including restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), real-time PCR, Vaccination with different types of vaccine provides a good expectation on disease control, such as vaccines produced in chicken-embryo-origin (CEO), tissue-culture-origin (TCO), and recombinant vaccines.

[Zeinab M. S. Amin Girh, Nagwa S. Rabie and Mona S. Zak. **Avian Infectious Laryngotracheitis.** *Rep Opinion* 2019;11(2):1-7]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). http://www.sciencepub.net/report. 1. doi:10.7537/marsroj110219.01.

Keywords: Avian; Infectious; Laryngotracheitis

Introduction

Avian Infectious Laryngotracheitis (AILT) is a highly contagious disease, of chickens which consider the primary host **(Bagust et al., 1986)** and it may affect pheasants while Starlings, sparrows, crows, pigeons and ducks seem to be resistant to the virus **(Guy and Garcia, 2008).** The causative agent is a pneumotropic virus of the family Herpesviridae, genus Il to virus. Taxonomically, this virus is classified as a Gallidherpes virus 1**(King et al., 2012)**. It causes severe lesions in the respiratory tract and great economic losses due to mortality, decreased egg production, weight loss, and susceptibility to infections with other aviary pathogens **(Guy and Garcia, 2008)**. The severe form causes significant respiratory distress, expectoration of bloody sputum, sneezing, and high mortality. The mild form is characterized by mucoid tracheitis, sinusitis, and low mortality **(Ou et al., 2012).** The virus is horizontally transmitted, and the primary virus replication sites are in the tracheal mucosa and conjunctiva, where it can cause inflammation, mucoidor serous discharge, cough, and dyspnea **(Coppo *et al*., 2013a)**. The virus can invade the trigeminal nerve during the lytic phase of infection, resulting in a latent infection that may remain throughout the life of the animal, and some stressors, such as placement with other birds and the onset of egg laying, can cause reactivation of replication and viral excretion **(Hughes *et al*., 1989; Hughes *et al*., 1991; Coppo *et al*., 2013a; Williams *et al*., 1992)**. New experimental studies show that the virus can also be detected in other organs, such as the heart, liver, spleen, lung, kidney, tongue, thymus, proventriculus, pancreas, duodenum, small intestine, large intestine, cecum, cecal tonsils, bursa, and brain **(Zhao *et al*., 2013; Wang *et al*., 2013; Oldoni *et al*., 2009; Parra *e t al*., 2015a).**

Etiology

The avian infectious laryngotracheitis virus belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Iltovirus*, and it is taxonomically classified as a *Gallidherpesvirus* type 1. The subfamily includes important human and animal pathogens, such as human herpesvirus 1 (Simplex virus); pseudorabies swine virus and varicella zoster virus (Varicellovirus), and other alphaherperviruses, such as that causes Marek's disease **(Johnson and Tyack, 1995).** This DNA virus has icosahedral symmetry, measures195- 250 nm in diameter, has a density of 1.704 g/mL, and molecular weight of approximately 1 x 108. Its genome consists of a linear double-stranded molecule of 155 kb, with a unique long region (UL) and a unique short region (US), flanked by inverted repeats **(Johnson *et al*., 1991; Bagust *et al*., 2000).**

Hosts

Chickens of all ages are susceptible to the infection by the laryngotracheitis virus, but birds older

than three weeks are more sensitive **(Ou et al., 2012)**. The virus can also infect pheasants, pheasants-chicken crosses, peacocks, and young turkeys **(Crawshaw and Boycott, 1982; Portz et al., 2008)**. Starlings, sparrows, crows, doves, ducks seem to be refractory to the virus **(Guy and Garcia, 2008)**. ILTV was also isolated from a guinea fowl in a farm with a history of respiratory disease **(Bautista, 2003).** Subclinical ITLV infection and seroconversion was demonstrated in ducks **(Yamada et al., 1980).**

Transmission

The ILTV is naturally transmitted by the upper respiratory tract and also by ocular route. Ingestion may also cause infection; however, the nasal epithelium needs to be exposed after virus ingestion **(Robertson and Egerton, 1981).** The main sources of transmission are clinically-affected chickens, latent carrier animals, contaminated dust, litter beetles, drinking water, and fomites **(Ou *et al*., 2011).** Recent studies have shown that the virus may remain in the biofilm water and subsequently be transmitted to susceptible birds **(Ou et al 2012).** Other possible sources of transmission are dogs, cats, and crows **(Kingsburry and Jungherr, 1958)**. Airborne transmission among farms is also very important for the spread of the virus **(Johnson *et al*., 2005)**.

Clinical signs

The disease has severe and mild forms. In the severe form, the main clinical signs are overt dyspnea and bloody mucus, moderate to severe conjunctivitis, and there may be high morbidity (90-100%) and mortality, which may be higher than 70%, but usually is in the range of 10%-20%. Also, inflammation and necrosis are observed with hemorrhage in the mucosa and in the respiratory tract **(Bagust *et al*., 2000; Garcia *et al*., 2014).** In the mild enzootic form, the observed signs are depression, decreased egg production, non-thriftiness, conjunctivitis, swelling of the infraorbital sinuses (almond eyes), mild mucoid tracheitis, respiratory rales, mild hemorrhagic conjunctivitis, and persistent nasal discharge; morbidity and mortality may reach5% and 0.1-2%, respectively **(Raggi *et al*., 1961; Ou *et al*., 2012).** Birds may recover in 10 to 14 days, but the clinical signs caused by the infection with some strains continue for a few weeks **(Guy and Garcia, 2008; Garcia *et al*., 2014; Ou *et al*., 2012).** The clinical signs appear after 6-12 days of natural exposure; however, in experimental infections. the incubation period is shorter, of around 4-7 dpi **(Garcia *et al*., 2014).**

Lesions

Gross lesions may be observed in the conjunctiva and the entire respiratory tract of infected birds, but are most frequently seen in the larynx and trachea. In the severe form, mucoid inflammation is observed in the early stages, and hemorrhage, degeneration and necrosis are observed in later stages. The inflammation may extend down to the bronchi, lungs, and air sacs. Diphtheritic changes, present as mucoid casts, may affect the entire length of the trachea **(Garcia *et al*., 2014)**. In the mild form, moderate mucoid tracheitis, presenting varying hemorrhage degrees in the larynx and upper trachea, is observed **(Sellers *et al*., 2004).** Microscopic lesions vary with the stage of the disease. In the first days, goblet cells and infiltration of the tracheal mucosa with inflammatory cells are increased. As the infection progresses, epithelial cells of the conjunctiva and respiratory tract become enlarged and edematous, and multinucleated cells form syncytia. Lymphocytes, histiocytic, and blood cells migrate into the mucosa and submucosa in 2-3 days **(Garcia *et al*., 2014)**. Intranuclear inclusion bodies in the tracheal and conjunctival epithelial cells are present for a few days (1-5 dpi) in the early stages of infection, and then disappear due to necrosis and sloughing of the epithelium **(Guy *et al*., 1992)**.

Diagnosis

Infectious laryngotracheitis is usually diagnosed in the laboratory because other diseases cause very similar clinical signs and lesions, such as avian influenza, bronchitis, Newcastle's disease, infectious coryza, and mycoplasmosis. The diagnosis based on clinical signs is only reliable in cases of acute severe disease, with high mortality and expectoration of blood **(Guy & Garcia, 2008).**

Histopathology

Infectious laryngotracheitis is characterized by the presence of eosinophilic intranuclear inclusion bodies, which are pathognomonic when present in the epithelial cells of the conjunctiva and of the respiratory tract. Those inclusion bodies are detected in the tissues by staining with Giemsa or with hematoxylin and eosin of tracheal section embedded in paraffin wax **(Guy & Garcia, 2008).** Epithelial hyperplasia leads to the formation of multinucleated cells (syncytia) in which intranuclear inclusions bodies may be evidenced. In addition, the tracheal tissue is infiltrated by heterophils and lymphocytes **(Fahey and York, 1990)**. Lamina propria swelling is observed after hemorrhage, as well as epithelial sloughing and loss of mucous glands. Tissue regeneration starts after approximately six days, after which intranuclear inclusions bodies are no longer visible **(Bagust *et al*., 2000)**. Rapid histopathology methods for tissue processing have been described, and include rapid dehydration of tissues to allow examination after three hours of processing **(Pirozok *et al*., 1957; Sevoian, 1960)**. Although ILT histopathological diagnosis by the detection of

intranuclear inclusion bodies is highly specific, virus isolation is more sensitive **(Guy *et al*., 1992).**

Virus isolation

The ILT virus can be isolated from clinical samples obtained from swabs, tissue homogenates, and trachea, larynx, lung, and conjunctiva exudates. The most sensitive isolation method is inoculation in the chorioallantoic membrane (CAM) of embryonated chicken eggs with 9 to 12 days **(Hichtner *and White* 1958)**. The virus causes the production of plaques with opaque edges and a central depressed area of necrosis **(Garcia *et al*., 2014).** The virus can also be isolated using cell cultures, particularly CEL and CK, although the CEL system is more sensitive. In cell cultures, multinucleated giant cells are detected 24 hpi. Both in CAM and cell culture systems, more than one passageis required virus isolation **(Bagust *et al*., 2000).** The samples should be collected as soon as possible after the establishment of clinical signs because isolation attempts are successful 6-7 days after infection **(Guy *et al*., 1992).**

Other techniques of virus detection

Other methods for ILTV detection include immunofluorescence (IF), immunoperoxidase (IP), virus neutralization (VN), enzyme-linked immunosorbent assay (ELISA), DNA hybridization techniques, electron microscopy (EM), and PCR **(Bagust *et al*., 2000).** The IF or IP are performed using sections or scrapings of the epithelium of affected birds. Viral proteins have been detected by IF for up to fourteen days after exposure **(Wilks and Kogan, 1979).** It was shown that immunofluorescence can detect antibodies against ILTV in tracheal tissues 109 dpi and that IP is more sensitive than the IF **(Guy *et al*., 1992).** Further studies have shown that ELISA, using monoclonal antibodies against ILTV, provides accurate ITLV detection, and it is faster and more accurate than IF or immunodiffusion in agar gel **(Jordan and Chubb, 1962)**. The use of direct electron microscopy is one of the fastest methods to detect the ILTV, but is not very sensitive and virus titers of at least 3.0 log10 per gram are required to identify the viral particles **(Bagust *et al*., 2000).** Some molecular methods for the detection of viral DNA can identify the virus faster, more accurately, and are highly sensitive. Molecular techniques include hybridization assays, dot-blot, and cloning of viral DNA, which are very sensitive for viral detection when viral isolation and ELISA results are negative **(Keam *et al*., 1991; Key *et al*., 1994).** There are also other methods like PCR, nested PCR, real-time PCR, multiplex PCR, in situ hybridization **(Nagy, 1992; Nielsen *et al*., 1998)**, and PCR followed by restriction fragment length polymorphism (RFLP) **(Chang *et al*., 1997; Kirkpatrick *et al*., 2006; Chacon *et al*., 2010)**. The detection of ILTV by PCR is more sensitive than virus isolation or electron microscopy and also allows detecting the virus in samples containing other viral agents **(Williams *et al*., 1994).** When there are outbreaks of the disease, viral detection by real-time PCR is more sensitive in comparison with histology, electron microscopy, isolation in embryonated eggs, and IF. However, because many laboratories do not have the capacity to perform real-time PCR, ILTV diagnosis is routinely made using histopathology, IF, and PCR **(Crespo *et al*., 2007).** A new method for detecting ILTV nucleic acid was recently developed: the loop mediated isothermal amplification (LAMP). A comparison of this method with the real-time PCR showed that both are highly specific and sensitive. However, as the LAMP method is faster, less expensive, and does not require a thermocycler compared with real-time PCR, it could be used for routine laboratory diagnosis and real-time PCR can be used for further verification **(Ou *et al*., 2012).**

Prevention

It is very important to prevent contact between unvaccinated birds with vaccinated birds or with those recovering from an outbreak, which requires good management and biosecurity practices, as well as outbreak control. Biosecurity measures include protocols and procedures to prevent the infection and transmission of birds by humans, insects, wild birds, and other animals **(Kingburry *et al*., 1958; Ou *et al*., 2012).** Recently were used to control of outbreaks geographic information systems that provided information from a region for making plans of biosecurity, quarantine, vaccination and the route where the slaughterhouse of animals and early diagnosis, proper vaccination and cooperation between government and industry are very important for the control of laryngotracheitis **(Dufor-Zavala, 2008; Chin *et al*., 2009).**

Vaccination

Vaccination is the best method to prevent infection, but vaccinated birds may become latent infected carriers and be the source of virus transmission to non-vaccinated flocks. For this reason, vaccination is recommended in endemic areas **(Andreasen *et al*. 1989)**. High levels of protection are obtained 15-20 weeks post-vaccination, with variables degrees of protection throughout the year (York *et al*., 1989; Fahey & York, 1990). The most frequently used vaccine strains are modified-live virus of Tissue Culture Origin (TCO) or Chicken Embryo Origin (CEO). There is no significant difference in bird immunity 10 weeks after vaccination when CEO and TCO vaccines are compared; however, after 20 weeks, CEO vaccines provide better protection than TCO vaccines **(Andreasen *et al*., 1989)**. The main administration routes of live vaccines are via spray and drinking water. Vaccination via drinking water

may not be very effective due to water quality issues on different farms and because birds may not receive the amount of virus required to induce protection. Successful vaccination against ILTV requires the contact of the vaccine virus with the nasal epithelium cells **(Robertson and Egerton, 1981).** On the other hand, spray vaccination may cause severe reactions, as some birds may receive an overdose and very small droplets can penetrate deep into the respiratory tract **(Clarke *et al*., 1980).** Some studies indicate that the virulence of modified live vaccines increases with bird-to-bird passage, and after the sixth passage, may produce severe clinical signs in challenged birds. Chicken-embryo origin vaccines (CEO) show a greater tendency to increase their virulence with bird-to-bird passage relative to those of tissue-culture origin (TCO) **(Guy *et al*., 1991;**

Kotiw *et al*., 1995). Recent epidemiological studies indicate that the ILT outbreaks reported around the world are mainly related to the use of live attenuated vaccines **(Menendez *et al*., 2014).**

In recent years, recombinant vaccines are available in the market, and include the insertion of ILT viral glycoproteins into viral vectors, such as poxvirus (FPV) **(Davison *et al*., 2006)** and turkey herpesvirus (HVT) **(Vagnozzi *et al*., 2012)**. A recombinant fowl pox vaccine, containing the gene encoding the glycoprotein B (gB), was shown to protect chickens against virulent ILTV strains **(Tong *et al*., 2011).** Another avian pox recombinant vaccine, including the gene for glycoprotein B (gB) and the UL32 gene, was effective against the challenge of a virulent ILTV strain applied in the wing **(Coppo *et al*., 2013a).** These recombinant vaccines do not cause latent infection or reversion to virulence. Despite being safer than live attenuated vaccines, their high cost and the fact that they must be injected have limited their use **(Ou et al.,, 2012).**

Studies have been conducted to develop new vaccines using gene deletions. Some viruses with deleted genes may retain their ability to induce immune response while not producing clinical signs or latency. ILT viruses with deletions of the genes gC **(Pavlova *et al*., 2010)**, gG **(Devlin *et al*., 2006),** gJ **(Fuchs *et al*., 2005)**, TK **(Han *et al*., 2002),** UL0 **(Veits *et al*., 2003),** and UL 47 (Helferich *et al*., 2007) showed attenuation and may be used for vaccine production. A gG-deficient ILTV strain administered to 3-week-old SPF chickens via drinking water and eye drop induced adequate immunity against challenge with a wild strain and may potentially be used for large-scale vaccination; however, further studies are needed before it is applied to commercial poultry flocks **(Devlin *et al*., 2008)**. Vaccines using ILTV as recombinant viral vector to express highly pathogenic genes (H5 and H7) of avian influenza have been tested and shown to protect chickens from both laryngotracheitis and avian influenza **(Pavlova *et al*., 2009).** A AILTV vector with the HPAI H5 gene inserted in the deleted region of the UL50 gene protected chickens against challenges with homologous and heterologous H5N1 and H5N2 viruses, respectively **(Pavlova *et al*., 2009)**. Another vaccine ILTV was developed using the gB gene combined with chicken IL-18 as a bicistronic vector and induced better protection of chickens challenged with ILTV that those containing only the gB gene as monocistronic vector **(Chen *et al*., 2010).**

Treatment

To date, no drug has shown efficacy in reducing the severity of lesions or relieving symptoms of ILTV. Antibiotics have no effect against the virus, but may control possible secondary bacterial infection **(Guyand Garcia, 2008).** However, if ILTV is diagnosed early in an outbreak, unaffected birds may be vaccinated, protecting them before they are exposed to the disease **(Garcia *et al*., 2014).**

References

1. Andreasen JR, Glisson JR, Gooddwin MA, Resurreccion RS, Villegas P, Brown J. Studies of infectious laryngotracheitis vaccines: Immunity in layers. Avian Diseases 1989;33:524–530.
2. Bagust TJ, Calnek BW, Fahey KJ. Gallid-1 herpesvirus infection in the chicken.3. Reinvestigation of the pathogenesis of infectious laryngotracheitis in acute and early post-acute respiratory disease. Avian Diseases 1986;30:179–190.
3. Bagust TJ, Jones RC, Guy JS. Avian infectious laryngotracheitis. Revue Scientifique et Tecnique-Office International des Epizooties 2000;19:483–492.
4. Bautista D. Isolation of infectious laryngotracheitis vٍrus (ILTV) from peafowls and chickens with a history of respiratory diseases. Proceeding of the 140th AVMA Annual Convention; 2003; Colorado Springs, Colorado. USA; 2003. p. 24.
5. Chacń JL, Mizuma MY, Piantino Ferreira AJ. Characterization by restriction fragment length polymorphism and sequence analysis of field and vaccine strains of infectious laryngotracheitis virus involved in severe outbreaks. Avian Pathology 2010;39:425–433.
6. Chang PC, Lee YL, Shien JH, Shieh HK. Rapid differentiation of vaccine strains and field isolates of infectious laryngotracheitis virus by restriction fragment length polymorphism of PCR products. Journal of Virology Methods 1997;66:179–186.

7. Chen HY, Zhao L, Wei ZY, Cui BA, Wang ZY, Li XS, et al. Enhancement of the immunogenicity of an infectious laryngotracheitis virus DNA vaccine by a bicistronic plasmid encoding glycoprotein B and interleukin-18. Antiviral Research 2010;87:235–241.
8. Chin RP, Garcıa M, Corsiglia C, Riblet S, Crespo R, Shivaprasad HL, et al. Intervention strategies for laryngotracheitis: impact of extended downtime and enhanced biosecurity auditing. Avian Diseases 2009;53:574–577.
9. Clarke JK, Robertson GM, Purcell DA. Spray vaccination of chicken using infectious laryngotracheitis virus. Australian Veterinary Journal 1980;56:424–428.
10. Coppo MJC, Noormohammadi AH, Browning GF, Devlin JM. Challenges and recent advancements in infectious laryngotracheitis virus vaccines. Avian Pathology 2013a;42:195–205.
11. Coppo MJC, Noormohammadi AH, Browning GF, Devlin JM. Challenges and recent advancements in infectious laryngotracheitis virus vaccines. Avian Pathology 2013a;42:195–205.
12. Crawshaw GJ, Boycott BR. Infectious Laryngotracheitis in peafowl and pheasants. Avian Diseases 1982;26:397–401.
13. Crespo R, Woolcock PR, Chin RP, Shivaprasad HL, Garcia M. Comparison of Diagnostics Techniques in an Outbreak of Infectious Laryngotracheitis from Meat Chickens. Avian Diseases 2007;51:858–862.
14. Davison S, Gingerich EN, Casavant S, Eckroade RJ. Evaluation of the Efficacy of a Live Fowlpox-Vectored Infectious Laryngotracheitis/Avian Encephalomyelitis Vaccine Against ILT Viral Challenge. Avian Diseases 2006;50:50–54.
15. Devlin JM, Browning GF, Gilkerson JR, Fenton SP, Hartley CA. Comparison of the safety and protective efficacy of vaccination with glycoprotein- G-deficient infectious laryngotracheitis virus delivered via eye-drop, drinking water or aerosol. Avian Pathology 2008;37:83–88.
16. Dufor-Zavala L. Epizootiology of infectious laryngotracheitis and presentation of a industry control program. Avian Diseases 2008;52:1-7.
17. Fahey KJ, York JJ. The role of mucosal antibody in immunity to infectious laryngotracheitis virus in chickens. Journal of General Virology 1990;71:2401–2405.
18. Fuchs W, Wiesner D, Veits J, Teifke JP, Mettenleiter TC. In Vitro and in Vivo Relevance of Infectious Laryngotracheitis Virus gJ Proteins That Are Expressed from Spliced and Nonspliced mRNAs. Journal of Virology 2005;79:705–716.
19. Garcıa M, Spatz S, Guy JS. Infectious Laringotracheitis. In: Saif YM, Glisson JR, Fadly AM, Mcdougald LR, Nolan LK, Swayne DE, editors. Diseases of poultry. Ames: Iowa State Press; 2014. p. 161–79.
20. Guy JS, Barnes HJ, Smith L. Increased virulence of modified-live infectious laryngotracheitis vaccine virus following bird-to-bird passage. Avian Diseases 1991;35:348–355.
21. Guy JS, Barnes HJ, Smith LG. Rapid diagnosis of infectious laryngotracheitis using a monoclonal antibody based immunoperoxidase procedure. Avian Pathology 1992;21:77–86.
22. Guy JS, Garcıa M. Laryngotraqueitis. In: Saif YM, Glisson JR, Fadly AM, Mcdougald LR, Nolan LK, Swayne DE, editors. Diseases of poultry. Ames: Iowa State Press; 2008. p. 137–152.
23. Han MG, Kweon, CH, Mo IP, Kim SJ. Pathogenicity and vaccine efficacy of a thymidine kinase gene deleted infectious laryngotracheitis virus expressing the green fluorescent protein gene. Archives of Virology 2002;147:1017–1031.
24. Helferich D, Veits J, Mettenleiter TC, Fuchs W. The UL47 gene of avian infectious laryngotracheitis virus is not essential for in vitro replication but is relevant for virulence in chickens. Journal of General Virology 2007;88:719–731.
25. Hitchner SB and White PG. A comparison of embryo and bird infectivity using five strains of laryngotracheitis virus. Poultry Science 1958;37:648–690.
26. Hughes CS, Gaskell RM, Jones RC, Bradbury JM, Jordan FT. Effects of certain stress factors on the re-excretion of infectious laryngotracheitis virus from latently infected carrier birds. Reserch in Veterinary Science 1989;46:274–276.
27. Hughes CS, Williams RA, Gaskell RM, Jordan FTW, Bradbury JM, Bennett M, et al. Latency and reactivation of infectious laryngotracheitis vaccine virus. Archives of Virology 1991;121:213–18.
28. Johnson MA, Prideaux CT, Kongsuwan K, Sheppard M, Fahey KJ. Gallid herpesvirus 1 (infectious laryngotracheitis virus): Cloning and physical maps of the SA-2 strain. Archives of Virology 1991;119:181–198.
29. Johnson MA, Tyack SG. Molecular evolution of infectious laryngotracheitisvirus (ILTV; gallid

herpesvirus 1): an ancient example of the Alphaherpesviridae? Veterinary Microbiology 1995;46:221–231.
30. Johnson YJ, Gedamu N, Colby MM, Myint MS, Steele SE, Salem M, et al. Wind-borne transmission of infectious laryngotracheitis between commercial poultry operations. International Journal of Poultry Science 2005;4:263–267.
31. Jordan FTW, Chubb RC. The agar gel diffusion technique in the diagnosis of infectious layngotracheitis (ILT) and its diferentiation from fowl pox. Reserch in Veterinary Science 1962;3:245–255.
32. Keam L, York JJ, Sheppard M, Fahey KJ. Detection of infectious laryngotracheitis virus in chickens using a non-radioactive DNA probe. Avian Diseases 1991;35:257–262.
33. Key DW, Gough BC, Derbyshire JB, Nagy E. Development and Evaluation of a Non-isotopically Labeled DNA Probe for the Diagnosis of Infectious Laryngotracheitis. Avian Diseases 1994;38:467–474.
34. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ. Ninth Report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier Academic Press; 2012; p. 1344.
35. Kingsbury FW, Jungherr EL. Indirect transmission of infectious laryngotracheitis in chickens. Avian Diseases 1958;2:54-63.
36. Kirkpatrick NC, Mahmoudian A, O'Rourke D, Noormohammadi AH. Differentiation of infectious laryngotracheitis virus isolates by restriction fragment length polymorphic analysis of polymerase chain reaction products amplified from multiple genes. Avian Diseases 2006;50:28– 34.
37. Kotiw M, Wilks CR, May JT. The effect of serial in vivo passage on the expression of virulence and DNA stability of an infectious laryngotracheitis virus strain of low virulence. Veterinary Microbiology 1995;45:71–80.
38. Menendez KR, Garcیa M, Spatz S, Tablante NL. Molecular epidemiology of infectious laryngotracheitis: a review. Avian Pathology 2014;43:108– 117.
39. Nagy E. Detection of infectious laryngotracheitis virus infected cells with cloned DNA probes. Canadian Journal of Veterinary Research 1992;56:34–40.
40. Nielsen OL, Handberg KJ, Jorgensen PH. In situ hybridization for the detection of infectious laryngotracheitis virus in section of trachea from experimentally infected chickens. Acta Veterinaria Scandinava 1998;39:415–421.
41. Oldoni I, Rodrیguez-Avila A, Riblet SM, Zavala G, Garcیa M. Pathogenic and growth characteristics of selected infectious laryngotracheitis virus strains from the United States. Avian Pathology 2009;38:47–53.
42. Ou SC, Giambrone JJ, Macklin KS. Comparison of a TaqMan real-time polymerase chain reaction assay with a loop-mediated isothermal amplification assay for detection of Gallid herpesvirus 1. Journal of Veterinary Diagnostic Investigation 2012;24:138–141.
43. Ou SC, Giambrone JJ, Macklin KS. Comparison of a TaqMan real-time polymerase chain reaction assay with a loop-mediated isothermal amplification assay for detection of Gallid herpesvirus 1. Journal of Veterinary Diagnostic Investigation 2012;24:138–141.
44. Ou SC, Giambrone JJ, Macklin KS. Infectious laryngotracheitis vaccine virus detection in water lines and effectiveness of sanitizers for inactivating the virus. Journal of Applied Poultry Research 2011;20:223–230.
45. Parra SHS, Nuẽz LFN, Astolfi-Ferreira CS, Ferreira AJP. Ocurrence of Infectious Laryngotracheitis Virus (ILTV) in 2009-2013 in the State of Sەo Paulo – Brazil. Brazilian Journal of Poultry Science 2015a;17:117– 120.
46. Pavlova SP, Veits J, Blohm U, Maresch C, Mettenleiter TC, Fuchs W. In vitro and in vivo characterization of glycoprotein C-deleted infectious laryngotracheitis virus. Journal of General Virology 2010;91:847–857.
47. Pavlova SP, Veits J, Keil GM, Mettenleiter TC, Fuchs W. Protection of chickens against H5N1 highly pathogenic avian influenza virus infection by live vaccination with infectious laryngotracheitis virus recombinants expressing H5 hemagglutinin and N1 neuraminidase. Vaccine 2009;27:773–785.
48. Pirozok RP, Helmbolt CF, Jungherr EL. A rapid histological technique for the diagnosis of infectious avian laryngotracheitis. Journal of American Veterinarian Medical Association 1957;130:406–407.
49. Portz C, Beltrao N, Furian TQ, Jٞnior AB, Macagnan M, Griebeler J, et al. Natural infection of turkeys by infectious laryngotracheitis virus. Veterinary Microbiology 2008;131:57–64.
50. Raggi LG, Brownell JR, Stewart GF. Effect of infectious laryngotracheitis on egg production and quality. Poultry Science 1961;40:134–140.
51. Robertson GM, Egerton JR. Replication of infectious laryngotracheitisvirus in chickens following vaccination. Australian Veterinary Journal1981;57:119–123.

52. Sellers HS, Garcیa M, Glisson JR, Brown TP, Jean S, Guy JS. Mild Infectious Laryngotracheitis in Broilers in the Southeast. Avian Diseases 2004;48:430–436.
53. Sevoian M. A quick method for the diagnosis of avian pox and infectious laryngotracheitis. Avian Diseases 1960;4:474–77.
54. Tong GZ, Zhang SJ, Meng SS, Wang L, Qui HJ, Wang YF, et al. Protection of chickens from infectious laryngotracheitis with a recombinant fowl pox virus expressing glycoprotein B of infectious laryngotracheitis virus. Avian Pathology 2001;30:143–148.
55. Vagnozzi A, Zavala G, Riblet SM, Mundt A, Garcیa M. Protection induced by comercially available live-attenuated and recombinant viral vector vaccines against infectious laryngotracheitis virus in broiler chickens. Avian Pathology 2012;41:21-31.
56. Veits J, Luschow D, Kindermann K, Werner O, Teifke JP, Mettenleiter TC, et al. Deletion of the non-essential UL0 gene of infectious laryngotracheitis (ILT) virus leads to attenuation in chickens, and UL0 mutants expressing influenza virus haemagglutinin (H7) protect against ILT and fowl plague. Journal of General Virology 2003;84:3343–3352.
57. Wang LG, Ma J, Xue CY, Wang W, Guo C, Chen F, et al. Dynamic distribution and tissue tropism of infectious laryngotracheitis virus in experimentally infected chickens. Archives of Virology 2013;158:659–666.
58. Wilks CR, Kogan VG. An immunofluorescence diagnostic test for avian infectious laryngotracheitis. Australian Veterinary Journal 1979;55:385–388.
59. Williams RA, Bennett M, Bradbury JM, Gaskell RM, Jones RC, Jordan FTW. Demonstration of sites of latency of infectious laryngotracheitis virus using the polymerase chain reaction. Journal of General Virology1992 73:2415–2420.
60. Williams RA, Savage CE, Jones RC. A comparison of direct electron microscopy, virus isolation and a DNA amplification method for the detection of avian infectious laryngotracheitis virus in field material. Avian Pathology 1994;23:709–720.
61. Yamada S, Matsuo K, Fukuda T, Uchinuno Y. Susceptibility of ducks to the virus of infectious laryngotracheitis. Avian Diseases 1980;24:930–938.
62. York JJ, Young JG, Fahey KJ. The appearance of viral antigen and antibody in the trachea of naive and vaccinated chickens infected with infectious laryngotracheitis virus. Avian Pathology 1989;18:643–658.
63. Zhao Y, Kong C, Cui X, Cui H, Shi X, Zhang X, et al. Detection of infectious laryngotracheitis virus by real-time PCR in naturally and experimentally infected chickens. PloS ONE 2013;8:1–10.

2/20/2019