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Review

Epidemiology of Avian Infectious Laryngotracheitis with Special Focus to South America: an update.

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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. It is caused by a *Gallid herpesvirus type 1*, a member of the genus *Iltovirus*. 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. Infected birds with clinical symptoms are main sources of transmission, but birds with latent infections, litter, and contaminated fomites may also transmit the virus. 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 RFLP, polymerase chain reaction (PCR), real-time PCR, and loop-mediated isothermal amplification. 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. However, in endemic areas, biosecurity measures and best management practices are important for the control of the disease. It is distributed worldwide and, in South America, it has been reported in Brazil, Peru, Ecuador, Bolivia, and Argentina causing great economic losses.

INTRODUCTION

Avian Infectious laryngotracheitis (AILT) is a highly contagious disease. Chickens are the primary host (Bagust *et al*., 1986) but it may affect pheasants and chicken. Starlings, sparrows, crows, pigeons and ducks seem to be resistant to the virus (Guy & Garcia, 2008). The causative agent is a pneumotropic virus of the family *Herpesviridae*, genus *Iltovirus.* Taxonomically, this virus is classified as a *Gallidherpesvirus* 1 (King *et al.*, 2012). This disease is included in the OIE list of mandatory notification of diseases of terrestrial and aquatic animals, as well as for the Brazilian Official Service (http://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2014/). 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 & 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 & Giambrone, 2012).

The disease was first described in 1925 (May & Tittsler, 1925), and has been reported in many countries in which remains an endemic disease, especially in areas of intensive poultry production, with great concentration of poultry farms rearing birds with multiple ages, including North America, China, Europe, Australia, Africa, Southeast Asia, New Zealand, Australia, Poland, South America, and Brazil (Hidalgo, 2003; Chacón & Ferreira, 2009).

The virus is horizontally transmitted, and the primary virus replication sites are in the tracheal mucosa and conjunctiva, where it can cause inflammation, mucoid or 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 *et al*., 2015a).

The importance of the Avian Infectious Laryngotracheitis virus for poultry demands constant update of our knowledge on this respiratory disease. This review article discusses virus characteristics, pathology, immunology, diagnosis methods, description of diseases and the main control strategies, with special focus on South America.

DESCRIPTION OF THE AGENT AND DISEASE

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 & Tyack, 1995).

This DNA virus has icosahedral symmetry, measures 195- 250 nm in diameter, has a density of 1.704 g/mL, and molecular weight of approximately 1 x 10^8. 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).

Susceptibility to chemical and physical agents

The ILTV is sensitive to lipolytic agents, such as ether and chloroform, but can survive for several months stored in suitable diluents, such as glycerol (50%) and sterile skimmed milk at 4°C (Bagust *et al*., 2000). The virus is also able to survive in chicken tracheal exudate and carcasses for 10-100 days at room temperature between 13-23 °C (Jordan, 1966). Chemical disinfectants derived from coal tar, formaldehyde, hypochlorite, and iodophor can effectively inactivate the virus (Guy & Garcia, 2008). In addition, the virus may be inactivated by solutions of 3% or 5% cresol in less than one minute and the use of 5% hydrogen peroxide results efficient disinfection of poultry equipment (Neighbour *et al*., 1994). Commercially available biofilm-reducing sanitizers are effective for removing residual vaccine virus from water lines and nipple drinkers after consecutive flock vaccination via drinking water (Ou *et al*., 2011).

Laboratory host systems

The laryngotracheitis virus can be propagated in embryonated eggs, which induce the formation of opaque plaques on the chorioallantoic membrane (CAM) observed 48 hours post-infection (hpi), and embryonic death occurs between 2-8 days post-infection (dpi). ILTV has been propagated in a variety of avian cell cultures, such as chicken embryo liver (CEL), chicken embryo kidney (CEK), and chicken kidney cell cultures (CK). The virus causes rounding of the nucleoli, chromatin displacement, increase of refractiveness, swelling of cells, as well as syncytium formation as the result of cytoplasmic fusion (Hughes & Jones, 1988; Garcia *et al*., 2014). Chicken embryo liver cells are the most susceptible for the primary isolation of the virus from clinical material (Garcia *et al.*, 2014). On the other hand, chicken embryo fibroblast cells, Vero cells, and quail cells have little susceptibility to the growth of virus derived from field material (Schnitzlein *et al*., 1994; Garcia *et al*., 2014). Furthermore, the virus may be propagated in LMH (leghorn male hepatoma) cells, a cell line derived from a chemically-induced chicken liver tumor, and it is used by research laboratories for the study of virus-host interactions (Schnitzlein *et al*., 1994).

Hosts

Chickens of all ages are susceptible to the infection by the laryngotracheitis virus, but birds older than three weeks are more sensitive (Ou & Giambrone, 2012). The virus can also infect pheasants, pheasants-chicken crosses, peacocks, and young turkeys (Crawshaw & Boycott, 1982; Portz *et al*., 2008). Starlings, sparrows, crows, doves, ducks seem to be refractory to the virus (Guy & 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 of 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 & 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 & Giambrone, 2012). Other possible sources of transmission are dogs, cats, and crows (Kingsburry & Jungherr, 1958). Airborne transmission among farms is also very important for the spread of the virus (Johnson *et al*., 2005).

Acute and latent infection

The target organ for infection and disease development is the respiratory epithelium. The trachea and larynx epithelia are the most affected, although other mucous membranes, such as the conjunctiva, nasal sinuses, air sacs, and lung tissue can be occasionally infected. When the birds are exposed to the virus by oral, nasal, or conjunctival route or by experimental inoculation in the breast, the virus replicates in the tracheal epithelium. Viral replication occurs only during the first week after infection, although low levels of the virus may be sporadically detected ten days after infection (Bagust, 1986; Williams *et al*., 1992). From approximately ten days to four weeks after infection, tracheal spread of the infection can be stopped, but a latent phase of infection may be established by the invasion of nervous tissues. Trigeminal nerve invasion by the virus can occur between days three and six in the acute phase of infection by either field or vaccine strains (Bagust, 1986). The precise route of infection of the trigeminal ganglion is unknown, but this nerve provides sensory innervation to the tissues of the upper respiratory tract, tongue and eyes, and the distal part of the nerve is also involved in the innervation of the trachea (Bagust, 1986; Williams *et al*., 1992; Bagust & Johnson, 1995). Studies using PCR indicate that the trigeminal ganglion is the main organ of virus latency (Williams *et al*., 1992). The virus can be reactivated from the trigeminal nerve 15 months post-vaccination and the viral DNA can be detected two days later in birds vaccinated by ocular route (Rodriguez-Avila *et al*., 2007; Williams *et al*., 1992). Virus shedding may considerably increase when birds are subjected to stress, such as the beginning of the laying period or when are mixed with unknown birds. In this case, birds with latent infection may act as reservoirs and cause infection in susceptible animals (Hughes *et al*., 1991; Williams *et al*., 1992).

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 reach 5% 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 & 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).

Immune response to ILTV

A variety of immune responses are generated after infection of ILTV (Garcia *et al.*, 2014). Humoral immunity is not the main response against the virus (Ou & Giambrone, 2012). In humoral response tests, such as virus neutralization and ELISA, antibodies are detected after 5-7 dpi, peak on 21 dpi, and may decline in the next months (Jordan, 1981) or persist for a year or longer (Bagust, 1986). Antibodies can be detected in tracheal secretions approximately 7 dpi and peak 10-28 dpi (Garcia *et al*., 2014).

The cell-mediated immune response is considered of great importance in the resistance against AILTV (Coppo *et al*., 2013b). Experiments have shown that birds bursectomized with cyclophosphamide and surgical methods the block their humoral immune developed cell-mediated immune response to the AILTV (York & Fahey, 1990).

Studies suggest that the gG gene present in the ILTV genome functions as broad-spectrum viral chemokine binding protein (vCKBP). This protein presents similar characteristics as some poxviruses and the murine gamma herpesvirus 68 (MHV-98), and their function is to evade host immune responses (Coppo *et al*., 2013b). Maternal antibodies are transmitted to offspring via eggs (Benton *et al*., 1960), as shown by their presence in day-old chicks (Gharaibeh *et al*., 2008); however, they do not protect against infection or interfere with vaccination (Fahey *et al*., 1983).

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 & 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 (Guy *et al.*, 1991; Kotiw *et al.*, 1995). 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 & Giambrone, 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).

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 & 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 *et al*., 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 passage is 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 & 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 & 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).

Differential Diagnosis

Infectious laryngotracheitis need to be differentiated from other respiratory diseases, such as avian pox diphtheria, Newcastle's disease, avian influenza, and those cause by pathogens, including the infectious bronchitis virus, fowl adenovirus, and *Aspergillus* spp (Garcia *et al.*, 2014).

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).

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 (Guy & 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).

ILT STATUS IN SOUTH AMERICA

Brazil

In Brazil, ILTV was isolated and identified in chickens with respiratory signs, severe hemorrhagic tracheitis, and high mortality in Petrópolis, state of Rio de Janeiro (Hipólito *et al*., 1974) in 1974, and in 1980, the virus was again isolated and characterized as a low-pathogenicity virus for broilers (Soares *et al*., 1980). The first epidemics was reported in 10-month-old laying hens, which presented egg-production drop and mortality in 1981-1982 in the state of Rio de Janeiro (Araujo *et al*., 1982). In 1995, Vargas (1995) detected antibodies against ILTV in layer farms in the state of Rio Grande do Sul (Vargas, 1995). By the end of 2002, suspected outbreaks of infectious laryngotracheitis were reported in commercial layers in the region of Bastos and Tupã cities in the state of São Paulo (Ito *et al.,* 2003). The virus was isolated in embryonated eggs by Lanagro-SP Laboratory, detected by PCR and molecularly characterized at the University of São Paulo (Chacon *et al*., 2007; Chacon & Ferreira, 2008; Chacon & Ferreira, 2009). At that time, the control measures to prevent the spread of the disease by the farmers, under the coordination of health authorities, were quarantine and also started vaccination with live vaccines CEO and TCO vaccines. The disease had great impact on the region, where more than a million of laying hens died as an outcome of severe injuries in the trachea

(Chacon & Ferreira, 2008). The epidemics affected all farms in the region, but at different intensities, possibly due to different levels of biosecurity applied in the poultry farms. Broiler production was the most affected (Chacon *et al*., 2007; Chacon *et al*., 2015). The use of PCR-RFLP and analysis of sequences of the ICP4 gene of field isolates of the 2002-2003 clinical outbreak in Bastos showed that the outbreak was caused by a highly virulent non-vaccine strain, which continues circulating in the region of Bastos, despite the vaccination program implemented (Chacon *et al*., 2010; Parra *et al*., 2015b). In 2012, a new vaccine vector against infectious laryngotracheitis (FP Vectormune LT), developed by CEVA Animal Health, was presented to the poultry farmers of the region of Bastos (http://www.portalsuinoseaves.com.br/ceva-lanca-nova-vacina-contra-laringotraqueite-em-aves/). This is a vaccine vector using the pox virus genetically modified to express key protective antigens against the ILT virus. (http://www.ceva.com.br/Especies-Produtos/Lista-de-Produtos/VECTORMUNE-FP-LT).

Another vaccine available in Brazil is a recombinant vaccine, using as vector HVT (herpesvirus of turkeys) encoding ITLV glycoprotein genes I (gI) and gD of (Innovax®-ILT; Intervet International BV, Whitehouse Station, NJ, USA) (Couto, 2014).

In the state of Minas Gerais, there was an outbreak in the 2010 in multi-age laying hen farms (Preis *et al*., 2013), caused by a low-virulence field strain. The Brazilian authorities authorized the use of only vectored vaccines in this region for the prevention of new cases (Couto *et al*., 2014). Recent studies show that both the CEO and the TCO vaccine viruses are still circulating in commercial layer flocks in different regions of Brazil (Chacon *et al*., 2015; Parra *et al*., 2015b).

Argentina

In Argentina, there are periodic ILT outbreaks, especially in areas with high density of industrial poultry farms with poor management and biosecurity measures, in farms that rear both broilers and layers, and, in some cases, also backyard birds. These outbreaks are usually caused by the transmission of the virus from infected birds exposed to field virus or from birds vaccinated with CEO vaccines. The use and marketing of such vaccines have currently been suspended in Argentina by a provision of the Directorate of Agricultural Chemicals, Pharmaceuticals and Veterinary SENASA No. 1559/2007 http://www.senasa.gov.ar/Archivos/File/File2821-laringotraqueitis-infecciosa.pdf) (Back & Leão, 2003).

Peru

Infectious laryngotracheitis was reported for the first time in Peru in August 2008 (Leisequia, 2013), and caused high morbidity and mortality. The affected poultry farms were in the Department of Lima, and ILT was later reported in the departments of Arequipa, Ancash, Ica, La Libertad, and Tacna (OIE Wahid Interface, 2010). The main lesions described in laying hens and fighting roasters were inflammation of the nasal and paranasal sinuses, serum hemorrhagic or hemorrhagic discharge, inflammation of the eyelids and conjunctivitis, presence of caseous material in the palate, diphtheritic plaques on the esophagus and larynx, hemorrhagic clots in the trachea. In broilers, inflammation of the eyelids and conjunctivitis and bleeding in the trachea were the most common findings. Different vaccination programs with a vaccine vector were used on different farms and had a clear positive impact and benefit on the control of the disease (http://amevea-ecuador.org/datos/LARINGOTRAQUEITIS%20INFECCIOSA%20LA%20EXPERIENCIA%20PERUANA.pdf).

Ecuador

In the first half of 2012, infectious laryngotracheitis was reported by the first time in Ecuador. The virus was detected by PCR in the cities of La Concordia, Píllaro, and Salcedo, but the source of infection was unknown (unpublished data). The University San Francisco de Quito carried out a detailed investigation between March 2011 and March 2012. Samples were collected in the provinces of Pichincha, La Concordia, Tungurahua, Cotopaxi, Manabí, Guayas, and Chimborazo, and PCR-positive results were obtained in samples from the provinces of Cotopaxi, Tungurahua, and La Concordia. The results were delivered to the governmental agency AGROCALIDAD (Agencia Aseguradora de la Calidad del Agro) in May 2012. This agency collected its own samples and confirmed the results. AGROCALIDAD also evaluated population density, history of respiratory symptoms compatible with the disease, bird age and type of production (http://www.veterinaria.org/revistas/vetenfinf/nfondevila/ltiecuador2012.htm).

Bolivia

The most common poultry diseases in the valleys of Cochabamba are mycoplasmosis, salmonellosis, with a minor incidence of bronchitis. In the first half of 2005, four cases of laryngotracheitis were recorded (http://www.midiatecavipec.com/avicultura/avicultura020908.htm). ILTV was also reported in

Uruguay, Colombia and Chile (Hidalgo, 2003; Back & Leão, 2003).

Colombia

ILT was confirmed for the first time in the Department of Valle del Cauca in 1971 in chickens with respiratory distress, coughing, conjunctivitis, and lacrimation. The chickens were necropsied at the Diagnostic Center ICA in Cali and presented the following lesion: beak with bloody and mucoid exudate, different degrees of laryngitis and tracheitis, bloody serous exudate in the trachea of some birds and others with cheesy yellowish exudate, tracheal mucosa covered with a pseudomembrane composed of fibrin and necrotic tissue partially or completely occluding the tracheal lumen. The disease was confirmed by medical history, macroscopic and microscopic (histopathology) lesions, and viral isolation (Morales, 1971) in chicken embryonated eggs with 10- to 12-day-old via MCA. The virus identification was confirmed by serum neutralization tests (Villate, 1971). In 1978, the Animal Health services of Colombia, which are in charge of the Colombian Agricultural Institute, ICA, under the Ministry of Agriculture, prepared a document containing general country information (location, size, climate, land use) farm; animal health status in Colombia; the organization of the Animal Health Division of (services: diagnosis, inspection of refrigerators, quarantine, health campaigns, FMD control, brucellosis), animal species, and main diseases, which included the laryngotracheitis; and animal health legislation (Estupiñan, 1978). In documents of OIE 2015, ILT was not reported in that year (http://www.cabi.org/isc/datasheet/79280).

Venezuela

In 1963, ILT was mentioned in a Poultry Conference in Maracay (Ayala Lopez, 1963) and in 1982, the use of vaccines against laringotraqueitis was mentioned (Quiroz *et al.*, 1982). According to the OIE, the disease was not reported in 2015 (http://www.cabi.org/isc/datasheet/79280).

Paraguay

The resolution 2400 issued in 2015 by the Paraguayan Animal Health and Quality Service (SENACSA, Servicio Nacional de Calidad y Salud Animal), belonging to the Ministry of Agriculturw of Paraguay updated the list of notifiable diseases and included infectious laryngotracheitis. That resolution provides that the notification of suspected of confirmed diseases in the list is mandatory by all farmers and veterinarians who work in the area of animal health or diagnosis (http://www.senacsa.gov.py/application/files/7714/4582/8020/SENACSA-RES-2400-2015.pdf).

Uruguay

A study between 2008 and 2009 on farms located in the areas of Montevideo, Canelones, and Lavalleja showed an ILT seroprevalence of 31.5% in unvaccinated broiler using a software, which applied applying Bayesian inference methodology Monte Carlo based on Markov Chains (MCMC). The study concluded that the results may be attributed to natural exposure to field virus and/or vaccine virus of birds in neighboring farms (Trenchi, *et al.*, 2012).

FINAL REMARKS

The presence of the ILT in South America is evidenced by the reported outbreaks and the economic losses caused in poultry production, confirming that infectious laryngotracheitis has worldwide distribution. For this reason, it is important that good management and biosecurity practices are developed, improved, and implemented for its prevention and control. Management practices, new vaccines (recombinants), and a strict biosecurity measures can prevent economic losses and may also reverse the latency of the virus in all South American countries. It is important also to be updated of new information on specific clinical signs, epidemiology of disease, and associated diseases, especially immunosuppressive diseases, as well as on the immune status of flocks to prevent new outbreaks.

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