Infectious Laryngotracheitis Virus from Field Outbreaks in Layer Birds: A Review

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Abstract

Infectious laryngotracheitis (ILT) herpes virus continues to cause outbreaks of respiratory disease in chickens world-wide and is an important respiratory disease of chickens. In India and Asian countries, sporadic cases of ILT occur in all classes of birds, starting from commercial laying hens and hobby/show/game chickens, broilers and heavy breeders. The epornitics of ILT tend to occur where there are large populations of unvaccinated birds, i.e., in concentrated areas of layer production. ILT virus (ILTV) belongs to Alphaherpesvirinae and the Gallid herpesvirus 1 species. The transmission of ILTV is via respiratory and ocular routes. Layers which are endemic infected with ILT virus occur only in some regions of countries or even in particular multiple-age production farms where modified live vaccines are actually used, even though these biological products, as well as wild ILTV strains, can establish latent infections. Vaccination is a common method to prevent ILT. However, field isolates and vaccine viruses can establish latent infected carriers. According to PCR-RFLP results, virulent field ILTVs can be derived from modified-live vaccines. Therefore, modified-live vaccine reversion provides a source for ILT outbreaks on chicken farms. Two recently licensed commercial recombinant ILT vaccines are also in use. Presently recombinant and gene-deficient vaccine candidates are in the developmental stages and offer some hope for the control of this disease. However, in ILT endemic regions, improved biosecurity and management practices are critical for improved ILT control especially in our country where the import of these type of vaccines are still in the bay.

Keywords: *Infectious laryngotracheitis virus; Infectious laryngotracheitis; Gallid herpesvirus-1; Polymerase chain reaction combined with restriction fragment length polymorphism; Recombinant laryngotracheitis vaccines.*

Introduction

Infectious Laryngotracheitis (ILT) is a viral respiratory tract infection of chicken which produces severe production losses due to mortality of infected layers, pullets and adult birds and/or decreased weight gain and egg production. Severe epizootic forms of ILT show a great respiratory distress, gasping, expectoration of bloody mucus, and high mortality. Mild forms of infection, sometimes enzootic, are characterized by mucoid tracheitis, sinusitis, unthriftiness, and low mortality. The ILT was first described in 1925 (May & Thittsler), and it has been described in many countries in which remains as a serious disease mainly in areas of intensive production and large concentrations of chicken such as North America, South America, Europe, China, Southeast Asia and Australia. Chicken flocks which are endemic infected with ILT virus occur only in some regions of countries or even

in particular multiple-age production sites (industrial or backyard flocks). However, serious disease outbreaks continue to occur periodically whenever ILT virus strains can move from persistently infected flocks to non-vaccinated birds. In these cases modified live vaccines must to be used, even though these biological products, (as wild ILTV strains also do it), can establish latent infections. The chicken is the primary natural host of ILT virus. Infectious Laryngotracheitis virus (ILTV) is classified as a member of the family Herpesviridae in the subfamily Alphaherpesvirinae. The virus is taxonomically identified as Gallid herpesvirus 1 (Roizman, 1982). Strains of infectious laryngotracheitis virus may vary considerably in their virulence and there was evidence that vaccine derived strains have become established in the field (Graham et al., 2000). This ability of ILTV vaccine strains to re-circulate may also be responsible for some outbreaks in susceptible birds, as passage in birds has been reported to result in increasing virulence (Guy et al., 1991), while stress in latently infected birds has also been demonstrated to be responsible for the re-excretion of ILTV (Bagust et al., 2000).

Morphology and Chemical Composition

Electron micrographs of ILTV-infected chicken embryo cell cultures demonstrate the presence of icosahedral viral particles similar in morphology to herpes simplex virus. Watrach et. al. (1963) described de hexagonal nucleocapsid of ILTV to be 80-100 nm in diameter. The nucleocapsid have icosahedral symmetry and are composed of 162 elongated hollow capsomeres (Cruickshank et. al., 1963; Watrach et. al., 1963). The complete virus particle including an irregular envelope surrounding the nucleocapsid has a diameter of 195-250 nm. The envelope contains fine projections representing viral glycoprotein spikes on its surface. The nucleic acid of ILTV is comprised of DNA having a buoyant density of 1.704 g/mL, similar to other herpesviruses (Plummer et. al., 1969). Laryngotracheitis virus DNA has been reported to have a guanine plus cytosine ratio of 45% (Plummer et. al., 1969). The DNA genome consist of a linear 155-kb double-stranded molecule comprised of unique long and short segments flanked by inverted repeats (Johnson et. al., 1991; Lieb et. al., 1987). The glycoproteins of the virus, like other herpesviruses, are responsible for stimulating humoral and cell-mediated inmune responses. Five major envelope glycoproteins with molecular weights of 205, 160, 115, 90, and 60 kD have been reported (York et. al 1990). They are the major immunogens of ILT virus. LTV glycoproteins gB, gC, gD, gX, gK and the unique gp60 have been sequenced (Bagust & Johnson, 1995). Viral replication. The virus initiates infection by attachment to cell receptors followed by fusion of the envelope with the host cell plasma membrane. The nucleocapsid is released into the cytoplasm and transported to the nuclear membrane; viral DNA is released from the nucleocapsid and migrates into the nucleus through nuclear pores. Transcription and replication of viral DNA occur within the nucleus (Guo et. al., 1993) Transcription of ILTV DNA occurs in a highly regulated, sequentially ordered cascade similar to that of other

alphaherpesviruses (Prideaux et. al.,1992). Approximately 70 virus-coded proteins are produced; several are enzymes and DNA-binding proteins that regulate viral DNA replication, but most are viral structural proteins. Viral DNA replication occurs by a rolling circle mechanism with the formation of concatemers which are cleaved into monomeric units and packaged into preformed nucleocapsids within the nucleus. DNA-filled nucleocapsids acquire an envelope by migration through the inner lamellae of the nuclear membrane. Enveloped particles then migrate through the endoplasmic reticulum and accumulate within vacuoles in the cytoplasm (Guo et. al., 1993). Enveloped virions are released by cell lysis or by vacuolar membrane fusion and exocytosis.

Antigenicity and Virulence of LTV Strains

ILTV strains vary in virulence for chickens (Cover & Benton, 1958) virulence for chicken embryos (Izuchi & Hasagawa, 1982), plaque size and morphology in cell culture (Russell & Turner, 1983), and plaque size and morphology on CAMs of embryonated chicken eggs (ECE) (Pulsford & Stokes, 1953). Naturally occurring LTV strains vary in virulence from highly virulent strains that produce high morbidity and mortality in exposed chickens to strains of low virulence that produce mildto-inapparent infections (Cover & Benton, 1958; Jordan, 1966; Pulsford, 1963; Pulsford & Stokes, 1953). Laryngotracheitis virus strains appear to be antigenically homogenous based on virus-neutralization, inmunofluorescence tests, and cross-protection studies. However, minor antigenic variation among strains has been suggested by the finding that some strains are neutralized poorly by heterologous antisera (Pulsford & Stokes, 1953; Russell & Turner, 1983; Shibley et. al.1962). Differentiation of ILTV strains of varying virulence, particulary wild-type and modified live-vaccine viruses, is an important practical problem. Several methods for differentiating ILTV viruses have been studied including analysis of virulence for chicken embryos (Izuchi & Hasagawa, 1982), restriction endonuclease analyses of viral DNA (Guy et al., 1989; Kotiw et al., 1982; Lieb et al., 1987), and DNA hybridization assays (Kotiw et al.,1986). Assessment of mortality patterns in embryonated chicken eggs was proposed as a biologic system for differentiating ILTV strains and mortality patterns correlated closely with virulence. Restriction endonuclease cleavage of viral DNA and electrophoretic separation of DNA fragments has been shown to distinguish different ILTV strains (Lieb et al., 1987). Restriction endonuclease analysis of LTV DNA has been used extensively in epidemiologic studies of field outbreaks to differentiate wild-type and modified live-vaccine viruses (Andreasen et al., 1990; Guy et al., 1989; Keeler et al., 1993; Keller et al., 1992). Reciprocal DNA:DNA hybridization using cloned DNA fragments also has been shown to discriminate ILTV strains (Kotiw et al., 1986).

Infectious Laryngotracheitis Virus Infection and Its Hosts

All ages of chickens are affected, but chickens older than 3 wk are most

susceptible to ILTV (Fahey et al.,1983). It has been shown that ILTV can infect pheasants, pheasant- bantam crosses, and peafowl. ILT can infect turkeys at about 100 d of age. Clinical signs of dyspnea and depression can be observed in infected turkeys. Other avian species are resistant to ILTV infection. Embryonating chicken eggs are the most common method for propagating ILTVs. In chicken embryos, ILTV forms plaques on the chorioallantoic membrane (CAM). The plaques can be observed 48 h after infection, and embryos can die in 2-12 d post infection (PI). Strains of ILTV showed different plaque size and morphology on the CAM. The ILTV can be isolated in primary cell cultures, such as chicken embryo liver (CEL), chicken embryo kidney (CEK), and chicken kidney (CK) cell cultures. The sensitivity of ILTV isolation and propagation from field samples vary depending on the type of cell cultures. CEL was the most sensitive for isolation, followed by CK. The CEK and chicken embryo lung cells were less sensitive. Chicken embryo fibroblasts, Vero cells, and quail cells were not satisfactory.

Latency of ILTV

As is the case of other herpesviruses, ILTV establishes latent infections, which have been demonstrated by the re-isolation of virus from the seventh week after infection by repeated tracheal swabbings (Bagust, 1986), and at 2 months after infection in tracheal organ cultures (Adair et al., 1985). Trigeminal ganglion (TRG) is the main site of latency of ILT virus. The TRG provides the main sensory innervation to the tissues of the upper respiratory tract, and then neural viral migration is strongly inferred. Extra tracheal spread of LTV to
trigeminal ganglia 4-7 days after tracheal exposure was detected in 40% of chickens exposed to a virulent Australian LTV strain (Bagust et al., 1986). Reactivation of latent LTV from the trigeminal ganglia 15 months after vaccination of a flock has been also reported (Kaleta et al.,1986). Williams et al. (1992), demonstrated that mature laying chicken inoculated intratracheally with a field strain of ILTV showed viral DNA by PCR in trigeminal ganglia at 31, 46 and 61 days post – inoculation. No clear evidence exist for a viremic phase of infections. Clinically in apparent LTV infection of the respiratory tract is a major feature of LT persistence. Komarov & Beaudette (1932) and Gibbs (1933) demonstrated that collecting laryngeal and tracheal swabs from recovered infected birds and then inoculating susceptible chickens, indicated a “field” carrier rate of approximately 2% up to 16 months after a disease outbreak. Other studies with tracheal organ cultures explanted from chickens experimentally infected with Australian wild-type LTV and vaccine strains have been showed latent tracheal infections for similar periods in 50% or more of infected chickens (Bagust, 1986; Turner, 1972). Mechanical transmission can occur by use of contaminated equipment and litter (Beaudette, 1937; Dobson, 1935; Kingbury & Jungherr, 1958).

Clinical Signs

Clinical signs generally appear 6-12 days following natural exposure (Kernohan, 1931; Seddon & Hart, 1935). Experimental inoculation via the

intratracheal route results in a shorter incubation period of 2-4 days (Benton et al., 1958; Jordan, 1963; Seddon & Hart, 1935). Characteristic clinical signs include nasal discharge and moist rales followed by coughing, gasping, sneezing, depression and conjunctivitis (Beach, 1926; Kernohan, 1931a). When severe epizootic forms of the disease occur, signs also include labored breathing and expectoration of blood-stained mucus; and upon gross examination of the trachea, severe hemorrhages and mucus plugs are characteristics (Beach, 1926; Hinshaw et al., 1931; Jordan, 1958; Seddon & Hart, 1935; Guy & Bagust, 2003). Clinical signs associated with mild enzootic forms include unthriftiness, reduction in egg production, eye secretion, conjunctivitis, swelling of infraorbital sinuses, persistent nasal discharge, and hemorrhagic conjunctivitis. The course of the infection varies with the severity of lesions. Generally, most chickens recover in 10-14 days, but extremes of 1-4 week have been reported (Beach, 1926; Hinshaw et al., 1931).

Gross Lesions

Gross lesions are most consistently observed in the larynx and trachea, even though the conjunctiva and other respiratory tissues could be also be affected. Tissue changes in tracheal and laryngeal tissues may be mild, with only excessive amount of mucus, conjunctivitis, sinusitis, and mucoid tracheitis (Davidson et al., 1988; Linares et al., 1994), or severe, with hemorrhage and/or diphtheric changes. In severe forms, degeneration, necrosis, and hemorrhage occur in later stages. Mucoid secretions extended along the entire length of the trachea may be present. In other cases, severe hemorrhage into the tracheal lumen may result in blood clots, or blood may be mixed with mucus and necrotic tissue. Inflammation may extend down the bronchi into the lungs and air sacs. Edema and congestion of the epithelium of the conjunctiva and infraorbital sinuses may the only gross lesion observed in mild forms of LT.

Microscopic Lesions

Early microscopic changes in tracheal mucosa include the loss of goblet cell and infiltration of mucosa with inflammatory cells. As the viral infection progresses, cell enlarge, lose cilia, and become edematous. Multynucleated cells (syncytia) are formed and lymphocytes, histiocytes, and plasma cells migrate into the mucosa and submucosa after 2-3 days. Later, cell destruction and desquamation result in a mucosal surface either covered by a thin layer of basal cells or lacking any epithelial covering; blood vessels within the lamina propia may protrude into the tracheal lumen. Hemorrhage may occur in cases of severe epithelial destruction and desquamation with exposure and rupture of blood capillaries. Intranuclear inclusion bodies are found in epithelial cells by 3 days p.i. (Purcell, 1971). Inclusion bodies generally are present only in the early stages of infection (1-5 days) (Guy et al., 1992; Vander Kop, 1993); they disappear as infection progresses, a result of the necrosis and desquamation of epithelial cells.

ILTV Strain Differentiation

It is not possible to identify different strains of ILTV by serological methods, because ILTVs have close immunodominant domains (Shibley et

al1962). The most common and effective molecular method for ILTV differentiation is PCR followed by RFLP. PCR-RFLP analysis of single or multiple viral genome regions can differentiate strains from various geographic areas and vaccine from field strains (Leib et al 1986; Keeler at al.1993; Neff et al 2008; Oldoni, 2009.) Restriction endonuclease analysis of ILTV DNA can differentiate vaccine strains from wild type strains(Guy et al1989). Moreover, PCR-RFLP analysis of the partial

ICP4 gene, gC gene, and TK gene can distinguish field strains from vaccines. However, some virulent isolates could not be separated from vaccine strains (Chang et al1997). Han et al (2001) analyzed multiple genes with PCR-RFLP combined with DNA sequence analysis of the gG and TK genes to differentiate vaccine and non-vaccine strains. Researchers demonstrated that multiple gene PCR-RFLP was more reliable to differentiate vaccines from field strains (Kirkpatrick et al 2006). A new reverse RFLP method was reported to separate vaccine from non-vaccine ILTV strains. This method combined real-time quantitative PCR and restriction enzyme digestion and calculated the change of cycle threshold number value between digested and undigested template DNA for examining the genotype of ILTVs (Callison et al 2009). Oldoni et al (2007) investigated ILTV isolates from commercial poultry that were collected between 1988 and 2005 using multiple gene PCR-RFLP analysis (ORFBTK, ICP4, UL47/gG, and gM/UL9). They were able to separate ILTVs into nine genetic groups. Group Ⅰ and Ⅱ comprised the USDA reference strain and TCO vaccine strains. Group Ⅳ isolates were identical to CEO vaccine strains, whereas group Ⅴ isolates, which had one PCRRFLP pattern different from the CEO vaccine strains were CEO-related isolates. Group Ⅵ, Ⅶ, Ⅷ, and Ⅸ were field ILTV strains with genomic types different from CEO and TCO vaccines.

According to these reports, most ILTV field isolates in the US might come from vaccine reversion. Differentiation of vaccine from field viruses is important since countries can initiate trade barrier for importation of chicken products from areas where virulent field viruses exist. Therefore, the more common mild ILT is commonly referred to a "vaccinal" ILT even though PCR-RFLP testing has not always been done. In Europe, 104 field isolates were collected during 35 years from eight different countries. These virus isolates were analyzed with PCR-RFLP targeting the TK gene and it was shown that they separate into 3 genetic groups. It was also shown that 98 of these field isolates had the same RFLP patterns as vaccine strains (Neff et al 2009). In Australia, PCR-RFLP was used to analyze ILTV gG, TK, ICP4, ICP18.5 and ORFB-TK genes in 20 strains. These isolates could be discriminated into five genetic groups. Some isolates were closely related to vaccine strains (Blacker et al 2011).

Immunity

According with Jordan (1981), several types of immune responses are involved after ILTV infection. Virus-neutralization antibodies can be detected within 5 – 7 days p.i., with peak at 21 days and then antibody waned to be detected to low levels over a year (Hitchner et al.,

1958). York et al. (1989) founded that total specific antibody against ILTV was detected in tracheal washings from day 5 p.i., Ig A antibody appeared at day 6 p.i., but neutralizing antibody could not be detected until day 14. In ILTV vaccinated chickens there was a substantial increase in the number of Ig A- and Ig G-synthesizing cells in the trachea by day 3 p.i. with a marked increase in the numbers of IgA-positive cells at day 7 p.i. (York et al., 1989). Secretory antibodies, including Ig A, are important to confer resistance to infection at mucosal surfaces, such as respiratory tract (Waldman & Ganguly, 1974). Mucosal Ig A antibody responses are also known to be elicited more efficiently by local rather systemic administration of antigen (Gerber et al., 1978). The role of cell-mediated immune (CMI) mechanisms in recovery from herpes infections is well established (Nash et al., 1985; Zarling, 1986). The susceptibility of chickens to ILTV declined with age and meat-type males are more susceptible than meattype females. It has been also demonstrated that high environmental temperatures (350 C) cause higher

Diagnosis of ILT

ILT infections must be differentiated from other respiratory diseases which present similar clinical signs and lesions. In these cases LT diagnosis must be assisted by laboratory methods ILTV isolation. Laryngotracheitis virus may be propagated in embryonated chicken eggs (ECE) and a variety of avian cell cultures. In embryonated chicken eggs the virus causes formation of opaque plaques on the CAM resulting from necrosis and proliferative tissue reactions. Plaques are observed as early as 2 days p.i. and embryo deaths occur 2-12 days later. Survival time of inoculated embryos decreases with additional egg passages (Brandly, 1937; Burnet, 1934). LTV has been propagated in a variety of avian cell cultures (CC) including chicken embryo liver (CEL), chicken embryo lung, chicken embryo kidney (CEK), and chicken kidney (CK) cell cultures (Chang et al. 1960/1977?; Hughes & Jones, 1988; Meulemans & Halen, 1978a; McNulty et al., 1985). Hughes & Jones (1988) demonstrated that CEL and CK cells were more efficient for LTV isolation and propagation, with CEK cells, chicken embryo lung cells, and CAM inoculation of embryonated chicken eggs being less sensitive. Viral cytopathic effects may be observed in cell culture as early as 4-6 hr p.i. with a high multiplicity of infection. These assays also were shown to provide rapid methods for detection of chickens latently infected with LTV.

Polymerase Chain Reaction (PCR)

These tests for detection of LTV DNA have been described by Shirley et al. (1990) and Williams et al. (1994). Electron microscopic examination. Rapid diagnosis of LT also has been accomplished using direct electron microscopic examination of tracheal scrapings (Hughes & Jones, 1988; Van Kammen & Spradbrow, 1976). Diagnosis is dependent upon visualization and morphologic identification of herpes viruses and, thus, is successful only when large numbers of virus particles are present in clinical samples. Hughes & Jones (1988) found that virus particles were observed only when clinical samples

contained a minimum titer of 3.5 log10 of infectious virus. Serology. Demonstration of LTV antibodies in serum can be done through different tests: agar gel immunodiffusion (AGID), virus neutralization (VN) in ECE or CC, indirect fluorescent antibody (IFA) test, and ELISA. Actually, ELISA offers ease of testing for large number Management procedures for prevention and control For intensive broiler production, the short growth cycle and high level of biosecurity measures on farms can reduce the need for prophylactic vaccination. The application of biosecurity measures will avoid exposing susceptible chickens via contaminated fomites. For control of an ILT outbreak, the most effective approach is a coordinated effort to obtain a rapid diagnosis, to establish a vaccination program, and prevent further virus spread. Vaccination in the face of an outbreak will both limit virus spread and shorten duration of the disease. Spread of LTV between farms can be prevented by appropriated biosecurity measures. Laryngotracheitis virus infectivity is readily inactivated outside the host chicken by disinfectants and warm temperatures, thus carryover between successive flocks in a house can be prevented by adequate cleanup.

Traditional Live Attenuated ILT Vaccines

Traditionally there have been two sources of live attenuated ILT vaccines. Vaccines attenuated by multiple passages in embryonating eggs (CEO) (Samberg et al., 1971), are higher effective. However, in many cases their use can result in lower performance and higher condemnation rates. Broilers are generally vaccinated with CEO vaccine by drinking water only in the face of an outbreak. Furthermore, CEO-derived vaccine strains of ILTV are generally indistinguishable from true field isolates of ILTV (Guy et al., 1990), providing diagnosticians and regulators with additional challenges. ILT vaccines generated by multiple passages in tissue culture (TCO) (Gelenczei & Marty, 1964) generally offer less protection as they are more highly attenuated and less immunogenic. TCO vaccines are commonly used in layer breeders and layers. Live attenuated ILT vaccines provide immunity when apply via infraorbital sinuses (Shibley et al. 1962), intranasal instillation (Benton et al., 1958), eye drop (Sinkovic & Hunt,1968), and orally through drinking water (Samberg et al., 1971). However, application of ILT vaccines by eye drop method appears to be more protective than application by water or spray (Fulton et al., 2000). Most vaccines when given by eye drop method had lower mean microscopic lesion scores and higher ELISA titers after one vaccination. In contrast to other mass application methods, eye drop vaccination in flock situations when applied correctly ensures that all birds in that flock have received vaccine. Careful Eradication Eradication of LTV from intensive poultry production sites appears to be highly feasible due to several biologic and ecologic properties of the virus. These properties include the high degree of host-specificity of the virus, the relative fragility of ILTV infectivity outside the chicken, and antigenic stability of ILTV genome (Bagust & Johnson, 1995). Furthermore, the chicken is the primary

host species as well as the reservoir host. Because ILTV strains are antigenically homogeneous a single LTV vaccine produces cross- protective immunity for all LTV strains. Considering that backyard and fancier chicken flocks are likely reservoirs of LTV, they must be included in any eradication effort (Mallinson et al., 1981). Eradication of LTV will be facilitated in the future The development of ILT genetically engineered vaccines, that induce protective immunity without induction of latently infected carrier chickens, it will be easier to initiate eradication programs (Bagust & Johnson,1995). Actually, a vaccine on the base of a live Fowl Poxvirus vector genetically modified to express key protective ILTV antigens is commercially available.

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