

Avian infectious laryngotracheitis

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Summary

Avian infectious laryngotracheitis (ILT) herpesvirus continues to cause sporadic cases of respiratory disease in chickens world-wide. Sources of transmission of ILT infection are three-fold, namely: chickens with acute upper respiratory tract disease, latently infected 'carrier' fowls which excrete infectious laryngotracheitis virus (ILTV) when stressed, and all fomites (inanimate articles as well as the personnel in contact with infected chickens). Infectious laryngotracheitis virus infectivity can persist for weeks to months in tracheal mucus or carcasses. Rigorous site biosecurity is therefore critical in ILT disease control. Furthermore, while current (modified live) ILT vaccines can offer good protection, the strains of ILTV used in vaccines can also produce latent infections, as well as ILT disease following bird-to-bird spread. The regional nature of reservoirs of ILTV-infected flocks will tend to interact unfavourably with widely varying ILT control practices in the poultry industry, so as to periodically result in sporadic and unexpected outbreaks of ILT in intensive poultry industry populations. Precautions for trade-related movements of chickens of all ages must therefore include an accurate knowledge of the ILT infection status, both of the donor and recipient flocks.

Keywords

Avian diseases – Avian infectious laryngotracheitis virus – Biosecurity – Epidemiology – Gallid herpesvirus 1 – International trade – Quarantine – Regional distribution.

Introduction

Avian infectious laryngotracheitis (ILT) is a major viral respiratory disease which is included within List B of the Office International des Epizooties (OIE) (44). While the distribution of this disease is world-wide (10), chicken flocks which are endemically infected with ILT virus (ILTV) often occur only in some regions of countries or even in particular (multiple-age) production sites, irrespective of whether these are industrial or backyard flocks. However, serious disease outbreaks continue to occur periodically whenever ILTV strains can move from persistently infected flocks into non-vaccinated chickens. Uncertainty regarding ILT disease status is increased by the irregular or emergency-only vaccination for ILT in some countries. Furthermore, all currently available ILT vaccines are manufactured with modified live strains, which can also establish latent infections and cause disease when allowed to spread from bird to bird. Hence, close attention must be given to establishing the ILT

infection or vaccination status of chickens when birds are being moved, either nationally or internationally.

Description of the disease

Infectious laryngotracheitis (also referred to as laryngotracheitis or LT in the United States of America [USA]), is a viral respiratory infection of chickens that may result in severe economic losses as a result of mortality and/or decreased egg production. Although ILT is distributed world-wide, the disease may be present only in certain localities within a country or geographic region. The greatest incidence of disease is generally seen in areas of highly intensive poultry production.

Clinical signs characteristic of ILT include nasal discharge, moist rales, coughing and gasping (9). Two forms of the

disease are recognised, a severe epizootic form and a mild enzootic form.

Severe epizootic forms of ILT are characterised by marked dyspnoea, gasping, coughing, expectoration of blood-stained mucus and high mortality (9). Clinical signs associated with mild enzootic forms include unthriftiness, decreased egg production, watery eyes, conjunctivitis, swelling of infraorbital sinuses, nasal discharge and haemorrhagic conjunctivitis. Severe epizootic forms of ILT were common in earlier years, but from the 1950s and increasingly during recent years, mild enzootic forms have become more common, particularly in intensive poultry producing areas of Europe, Australia, New Zealand and the USA (39, 49).

Clinical signs generally appear between six and twelve days after natural exposure (49). The clinical course varies, but most chickens will recover in ten to fourteen days (9). Severe epizootic forms of ILT cause high morbidity (90%-100%) and variable mortality; mortality generally varies from 5% to 70% and averages 10%-20% (9, 49). Mild enzootic forms generally result in low morbidity (as low as 5%) and very low mortality (0.1%-2%) (39, 49).

Gross lesions may be found in the conjunctiva and throughout the respiratory tract of ILTV-infected chickens, but are most consistently observed in the larynx and trachea. Tissue changes in tracheal and laryngeal tissues may be mild, consisting only of excess mucus (39), or severe, with haemorrhage and/or diphtheritic changes. In mild forms of ILT, gross lesions may consist only of conjunctivitis, sinusitis and mucoid tracheitis (39). In severe forms, mucoid inflammation is observed early in infection with degeneration, necrosis and haemorrhage occurring in later stages. Diphtheritic changes are common and may be seen as mucoid casts that extend the entire length of the trachea. In other cases, severe haemorrhage into the tracheal lumen may result in blood casts, or blood may be mixed with mucus and necrotic tissue. Inflammation may extend down the bronchi into lungs and air sacs.

Oedema and congestion of the epithelium of the conjunctiva and infraorbital sinuses may be the only gross lesions observed in mild forms of ILT.

Microscopic changes vary according to the stage of the disease. Early microscopic changes in tracheal mucosa include the loss of goblet cells and infiltration of the mucosa with inflammatory cells. As the viral infection progresses, cells enlarge, lose cilia, and become oedematous. Multinucleated cells (syncytia) are formed and lymphocytes, histiocytes and plasma cells migrate into the mucosa and submucosa. Later, cell destruction and desquamation result in a mucosal surface either covered by a thin layer of basal cells or lacking any epithelial covering. Haemorrhage may occur in cases of severe

epithelial destruction and desquamation, with exposure and rupture of blood capillaries (16).

Intranuclear inclusion bodies may be found in epithelial cells of trachea, larynx and conjunctiva. Inclusion bodies are generally present only in the early stages of infection.

Aetiological agent

Classification

Infectious laryngotracheitis virus is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. The species is named gallid herpesvirus (42).

Virus morphology and structure

Infectious laryngotracheitis virus has the typical morphology of a herpesvirus. Virions have icosahedral symmetry and measure 100 nm-110 nm in diameter. The nucleocapsid contains 162 capsomers which are hexagonal in cross-section with a hole running half-way down the long axis. The core consists of a fibrillar pool on which the single molecule of double-stranded deoxyribonucleic acid (DNA) is wrapped. Surrounding each nucleocapsid is an irregular envelope of 120 nm-200 nm in diameter, bearing glycoprotein spikes on the outer surface (42).

Chemical composition

The chemical composition of ILTV has been reviewed by Bagust and Guy (7). The DNA has a buoyant density of 1.704 g/ml (consistent with other herpesviruses) and a molecular weight of approximately 100×10^6 . The guanine plus cytosine ratio is 45%. The double-stranded DNA comprises a linear 155 kilobase molecule of unique long and short segments flanked by inverted repeats. Five major envelope proteins have been reported to be the major immunogens of the virus.

Strain differentiation

Based on virus neutralisation (VN), immunofluorescence (IF) and cross-protection studies, ILTV strains are considered to be antigenically homogeneous. However, strains have been differentiated on the basis of virulence for chickens or chicks, plaque size and morphology in cell cultures and pock size on the chorioallantoic membranes (CAMs) of chicken embryos (7). Strains have been differentiated by restriction endonuclease analysis of viral DNA (15, 36, 38), and the use of the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) has enabled differentiation of field and attenuated vaccine-type strains (11).

Resistance to chemical and physical agents

In an *in vitro* environment, ILTV infectivity can be readily inactivated by low heat (e.g. 60°C for 15 minutes or less) or by freeze-thawing in a medium which is free of organic materials or protein. However, the presence of 50% glycerol broth or

sterile skim milk will greatly increase the time of survival possible for ILTV infectivity on swabs. Infectious laryngotracheitis virus in the laboratory is also susceptible to lipolytic solvents, such as ether or chloroform, and to extremes of pH (7).

In tracheal exudates and tissues, ILTV infectivity can persist for weeks or months in enclosed poultry production environments at relatively low temperatures (e.g. 10°C-20°C) (26, 30), this is discussed in greater detail below. However, putrefaction processes in the carcasses of dead chickens will shorten ILTV survival. Chemical disinfectants, such as coal tar derivatives, formalin, hypochlorite and iodophors, effectively inactivate ILTV on contact, as reviewed by Jordan (28) and Bagust and Guy (7).

***In vitro* cultivation**

Infectious laryngotracheitis virus can be grown in fertile chicken eggs and several avian primary cell cultures. Inoculation of the dropped CAM of eggs after incubation for ten to twelve days results in pock formation and embryo death between two and twelve days post inoculation (31).

The virus can be propagated in avian cell cultures derived from chick embryo liver (CEL), chick embryo lung, chick embryo kidney and chick kidney tissues, where the typical cytopathic effect is syncytium formation (22, 41). Chick embryo liver has been found to be the most sensitive system for primary isolation of virus from clinical material (22). Chick embryo fibroblasts, Vero cells and cells of quail origin have been shown to be relatively insensitive for ILTV growth from field material (22, 48).

Epidemiology

The primary host species for ILTV is the chicken, with natural transmission occurring by direct contact via the upper respiratory and ocular routes, mainly when infectious respiratory exudates are aerosolised or expectorated. No evidence exists for transmission of ILTV via the egg or for shedding of ILTV on shells of eggs laid by infected hens. The chicken is the only significant primary host species for ILTV, and no other reservoir host species is known to exist, although some other avian species, such as pheasants and peafowl (7), can sometimes be naturally infected by contact with chickens actively shedding ILTV.

The sources of ILTV are as follows:

- a) clinically affected fowls
- b) fowls which are latent 'carriers' of infection
- c) fomites and poultry farm personnel contaminated with ILTV.

Aspects which are important in understanding ILT infection and modes of spread of disease are discussed below.

Acute phase of infection

The target organ system for ILTV infection and disease is the respiratory tract. The epithelium of the trachea and larynx is always affected, whilst other mucous membranes such as the conjunctiva, as well as respiratory sinuses, air sacs and lung tissue, may also become infected periodically. Whether chickens are exposed to ILTV by nasal, oral, conjunctival, or even an experimental route such as via the intraorbital sinus, the most active replication of ILTV will occur within the tissues of the trachea. Active virus replication occurs only during the first week after infection, although low levels of ILTV infectivity can be detected sporadically, up to ten days post infection (p.i.) (5, 20, 54).

Chickens usually recover from primary ILT disease within seven to ten days of showing clinical signs, the appearance of these coinciding closely with active ILTV replication in the trachea. This limited period of active replication of ILTV has a critical bearing on prospects for achieving isolation of infectious virus from chickens with suspect ILT disease (see section entitled 'Diagnostic methods').

From ten days to approximately four weeks after tracheal infection, whilst shedding of infectious ILTV may have ceased, a latent phase of infection is established through ILTV invasion of nervous tissues. Invasion of the trigeminal ganglion (TRG) by ILTV has been found to occur regularly from days three to six, during the acute phase of ILT infections by field and vaccine strains (5). The exact route of infection of the TRG is not known, but neural migration is strongly inferred as this ganglion provides the main sensory innervation to the tissues of the upper respiratory tract, the mouth and the eyes, whilst the distal ganglia are also involved in the sensory innervation of the trachea. The use of PCR technology by Williams *et al.* (54) has confirmed that the tracheal ganglion is the main site of ILTV latency.

Latent infection with periodic reactivation and excretion of virus

Onset of the latent ('hidden') phase of ILTV infection commences from the immediate post-acute phase of infection, i.e. seven to ten days after tracheal exposure. Latent ILTV infections are not readily demonstrable during the first few months after infection (5, 21, 24), probably reflecting initially high levels of host immune control and surveillance. Subsequently, sporadic reactivation of latent ILTV infection with shedding of low levels of infectious virus into the trachea will recur throughout life.

A landmark study which helped to explain how apparently spontaneous outbreaks of ILT can occur in field situations (23), showed that rates of shedding of ILTV into the trachea could be significantly increased ($p < 0.001$) by the stresses of either the onset of lay or mixing with unfamiliar birds. In this case, the latently infected chicken can act as an unsuspected reservoir host and enable ILTV to infect further susceptible

chickens. It should be understood that establishment of latency by ILTV, in common with other herpesviruses, is the biological survival mechanism which enables ILTV to evade host immune surveillance and to persist, even in small flocks of chickens, over generations.

Immune responses

A variety of responses are generated by the immune system following infection by ILTV (7, 27). Best known are the virus-neutralising antibodies which become detectable in the serum within five to seven days of tracheal exposure, peak around twenty-one days, and then wane over the next several months to low levels at which they can persist for a year or more. Mucosal antibodies (immunoglobulin [Ig]G and IgA) which are capable of binding to ILTV antigen and low levels of virus-neutralising and enzyme-linked immunosorbent assay (ELISA) antibody activity become detectable in tracheal secretions and washings from approximately seven days p.i. (3, 57), and plateau at days ten to twenty-eight. However, numerous laboratory and field studies have independently confirmed that immune protection to ILTV challenge is neither indicated by, nor conferrable through, the presence of serum or maternally-derived antibody (7).

Cell-mediated immunity is known to be the protective immune response in ILT infection and for vaccination (27, 46). Studies by Fahey and York, using vaccinated-bursectomised chickens, have demonstrated that even tracheal mucosal antibody is not essential in preventing the replication of virus in vaccinated chickens (14). Rather, the effector mechanism of protection from ILT is likely to be the local cell-mediated immune response in the trachea.

Vaccines and biosecurity considerations

Primary vaccination with current (i.e. modified-live) ILT vaccine strains will confer partial protection against challenge by three to four days post exposure, and complete protection after one week (19, 27). High levels of protection occur between fifteen and twenty weeks post vaccination, with variable degrees of protection within a flock over the following year. Revaccination with infectious ('live') vaccines may or may not assist in maintaining protection levels (27), as the infectivity of any vaccine virus may be neutralised and replication prevented at the portal of entry into the host chicken (14, 57).

Whilst the chickens on a particular site may have been vaccinated adequately, it is important to remember that reactivation and excretion of ILTV will be occurring continually in a small proportion of these chickens. On mixed-age production sites, stressful events, such as entering into lay, or shifting and mixing of flocks will occur regularly, causing an even higher proportion of hens in a flock to shed virus into the environment. After exposure of a flock to 'live' ILT vaccine, or after an ILT outbreak, for practical purposes, active ILTV shedding cannot be assumed to have ceased until

approximately two weeks after vaccination or the onset of the last clinical cases in a naturally infected flock.

Field virus survival and inactivation

Clinically affected chickens are the major source of high levels of infectious virus through coughing and expectoration of ILTV-contaminated exudates onto the ground and the feathers of other birds and into the production environment. These exudates may passively contaminate the surroundings, including buildings, equipment of all descriptions, and very importantly, the skin, clothing and footwear of any personnel in contact. In the field, the presence of proteinaceous material enables ILTV infectivity to be maintained for much longer periods than would be expected from the fragility of the virus *in vitro*.

In a remarkable series of experiments designed to improve the understanding of ILTV epidemiology in the field, Jordan *et al.* showed that ILTV infectivity in tracheal mucus can survive at room temperatures (20°C-23°C) for up to three months on wooden surfaces if protected from light, and for up to twenty days in the deep litter of such poultry houses (30). Furthermore, ILTV within chickens which had died from ILT disease, was demonstrated to remain infectious for up to three weeks in buried carcasses. Separate studies on breaches of ILT control programmes (35, 40) have implicated ILTV spread between production sites occurring through dogs, rodents and even birds (crows) permitted to cannibalise improperly buried carcasses. Adequate site biosecurity and carcass disposal are therefore central measures in preventing the natural spread of ILTV.

For effective decontamination of poultry houses from ILTV, sheds must be sprayed with disinfectant (see below) and all potentially contaminated carcasses, feathers, feed, water and litter must be physically removed. Equipment should be washed with hot water and then soaked in virucidal disinfectants such as phenolics, sodium hypochlorite or iodophors (28), or a quaternary ammonium compound which has a wetting agent incorporated. All disinfectants should be used only at the dilutions recommended by the manufacturer.

Diagnostic methods

Although diagnosis of severe, acute ILT may be made on the basis of high mortality and expectoration of blood, the milder forms of the disease may resemble respiratory disease caused by other agents such as Newcastle disease and infectious bronchitis. Thus, for confirmation of diagnosis, laboratory methods are required. Those available are as follows:

- a) histological examination of the trachea
- b) detection of virus
- c) detection of antibodies.

Protocols for each of these techniques are given in detail in Tripathy (51) and in the OIE *Manual of Standards for Diagnostic Tests and Vaccines* (43).

Histological examination of the trachea

The development of eosinophilic intranuclear inclusion bodies in the respiratory and conjunctival epithelium is pathognomonic for ILT. Epithelial hyperplasia gives rise to multinucleated cells (syncytia) in which these intranuclear inclusions may be apparent. Additionally, an invasion with heterophils and lymphocytes occurs (45). Oedema is observed later, with haemorrhage of the lamina propria. Desquamation of the necrotic epithelium and loss of mucous glands also occur. Regeneration commences after approximately six days. After this time, intranuclear inclusions may not be visible. Thus, the appearance of these inclusions is transient and inability to see them does not exclude a diagnosis of ILT.

Inclusion bodies may be observed after staining with Giemsa or haematoxylin and eosin after embedding tracheae in paraffin wax. Longitudinal sections of tracheae may afford a greater opportunity to observe the inclusions. Rapid methods of processing tissues for histopathology have been described, and these involve the rapid dehydration of tissues to allow examination within three hours of processing (7). While histopathological diagnosis of ILT by detection of inclusion bodies is highly specific (33), sensitivity is poor when compared to virus isolation (17).

Detection of virus

A very simple method of ILTV detection is agar gel immunodiffusion (AGID) using a hyperimmune serum to ILTV (29). Virus is tested in macerated tracheal and laryngeal tissue from the affected bird, and although easy to perform, the test relies on a strongly positive antiserum. The AGID test may be of value in differentiating ILT from the diphtheritic form of fowlpox.

Isolation of ILTV may be conducted in embryonated eggs or cell cultures. Exudate and epithelial scrapings or tracheal swabs are emulsified in nutrient broth and the supernatants inoculated onto the dropped CAM of ten- to twelve-day-old fertile chicken eggs or into preformed monolayers of CEL cell cultures. Samples of tissue or swabs should be taken as soon as possible after the onset of clinical signs, since isolation attempts may be unsuccessful beyond six to seven days after infection (7). Inoculation of eggs with ILTV results in the production of pocks on the CAM. Inoculation of cell cultures causes syncytium formation. In both instances, more than one passage may be required before the virus is isolated. The virus should then be identified by an alternative means such as IF, VN or electron microscopy (EM). Hughes and Jones compared isolation of ILTV by inoculation of fertile eggs using three different routes, as well as primary chick and chicken cultures; CEL cells were found to be the most sensitive (22).

Identification by virus isolation is time consuming and laborious. Among the more rapid methods for detecting ILTV are IF or immunoperoxidase (IP), an antigen capture ELISA, EM, DNA hybridisation techniques and the PCR.

Immunofluorescence or IP are performed on sections or epithelial scrapings from affected birds. Wilks and Kogan detected viral proteins using IF for as long as fourteen days post exposure (53), but others found IF staining was positive over a shorter period (5, 20). Guy *et al.* reported that IP was more sensitive than IF (17). York and Fahey described a capture ELISA which is claimed to have the same accuracy as virus isolation whilst being more rapid, and to be more accurate than IF (56).

The use of direct EM for the examination of emulsified tracheal scrapings after negative staining provides potentially the most rapid method of detecting ILTV (22, 52). From receipt of material, observation of herpesvirus particles is possible in less than one hour. However, use of the EM is not very sensitive, and virus titres of $3.0 \log_{10}$ per gram may be required in the tissues for particles to be detected (22). Thus, EM should not be relied upon as a stand-alone detection method.

Methods for identifying ILTV DNA in clinical samples have been reported (31, 34, 50, 55). Dot-blot hybridisation assays and cloned virus DNA fragments were shown to be highly sensitive for detecting ILTV when isolation and ELISA were negative (31, 34).

For clinical samples, PCR is reported to be more sensitive than virus isolation (31, 50, 55), especially when other contaminant viruses such as adenoviruses are present (55). Alexander and Nagy found that in the middle of the post-infection phase, i.e. three to four days p.i., PCR and virus isolation were similar in sensitivity, but PCR was superior in the recovery phase tested between five to eight days p.i. (2). A problem of detection methods for ILTV has been that hitherto, differentiation between field strains and vaccine strains has not been possible. This problem would now seem to have been overcome by Chang *et al.* who used PCR in conjunction with RLFP (11). This approach offers considerable promise for improving understanding of both the epidemiology and evolution of ILTV.

Detection of antibodies

Several techniques have been used for monitoring of antibodies to ILTV in chicken serum, including AGID, VN, indirect immunofluorescence (IIF) and ELISA (1).

For AGID, antigen is provided by virus-infected CAMs or cell cultures (29). The advantage of the test is that it is very simple and can therefore be performed in any laboratory. However, the test is relatively insensitive. Virus neutralisation tests may be performed using fertile chicken eggs inoculated by the

CAM route with enumeration of the pocks on the CAM. Alternatively, the test may be performed more conveniently in cell cultures in microwell plates.

Antibodies to ILTV by IIF can be demonstrated by application of test sera to fixed, pre-infected cell cultures. The method is sensitive but relies on the intensity of fluorescence perceived by individuals, and hence interpretation of results may be subjective (1).

Enzyme-linked immunosorbent assay offers ease of testing for large numbers of sera, with semi-automation. This method has been demonstrated to be more sensitive than VN (1, 8), and of comparable sensitivity to IF, with AGID being the least sensitive (1). Enzyme-linked immunosorbent assay would therefore seem to be the system of choice for flock testing.

Public health implications

No public health implications of ILT have been recognised.

Prevention and control methods

In areas of intensive poultry production, ILT is usually controlled by a combination of biosecurity measures and vaccination using modified-live vaccines. This is particularly true for layer flocks and broiler breeder flocks; however, for intensive broiler production, a combination of strict biosecurity and the short growth cycle can obviate the need for prophylactic vaccination.

Given that ILTV infections resulting from field exposure or vaccination will result in latently infected carrier birds, it is extremely important to avoid mixing vaccinated or recovered birds with susceptible chickens. Special precautions should be taken to obtain a complete history when mixing breeding stock.

The importance of site quarantine and hygiene in preventing the movement of potentially contaminated personnel, feed, equipment and birds is central to successful prevention and control of ILT. In addition, the threat posed by backyard and exhibition poultry flocks should be recognised and guarded against (40).

Co-operative control of ILT outbreaks by collaboration between government and industry is most desirable. If implemented correctly, this approach may obviate the need for widespread use of ILT vaccine in control programmes (40). Where outbreaks have been contained, recovered flocks should be moved for processing under quarantine as soon as possible. Studies indicate that this interval can be as short as two weeks after the last clinical signs of ILT are observed on a site (13).

For control of an ILT outbreak, the most effective approach is a co-ordinated effort to obtain a rapid diagnosis, institute a vaccination programme and prevent further virus spread (4). Vaccination in the event of an outbreak will both limit virus spread and shorten duration of the disease. Spread of ILTV between sites can be prevented by appropriate biosecurity measures. The virus is readily inactivated outside the host chicken by disinfectants and low levels of heat, thus spread of disease between successive flocks housed in the same building can be prevented by adequate cleaning precautions.

Vaccination has proven to be a satisfactory method of developing resistance in susceptible chicken populations. However, since vaccination can potentially result in a variety of adverse reactions, this strategy is recommended for use only in geographic areas where the disease is endemic. Adverse reactions associated with use of modified-live ILT vaccines can include the production of latently infected carriers, potential for spread to non-vaccinated flocks and insufficient attenuation (3, 16).

At present, only modified-live vaccine viruses are available for prophylactic vaccination. Modified-live virus vaccines are prepared by propagation of vaccine strains, i.e. laboratory-attenuated viruses or naturally occurring avirulent strains, in cell culture (tissue culture-origin) or embryonated eggs (chick embryo-origin). When used correctly, these modified-live vaccines are highly effective in controlling ILT disease when the virus is later introduced into susceptible chicken populations (19).

The procedures of vaccine administration must be performed carefully to ensure adequate vaccination. An adequate concentration of infectious virus must be maintained to provide effective vaccination of chickens (18). Infectious laryngotracheitis vaccines are generally administered to chickens by eye drop, orally (through drinking water), or by spray. However, problems have been associated with both the drinking water and spray routes of inoculation. Successful vaccination via drinking water requires that vaccine virus contacts susceptible nasal epithelial cells as a result of aspiration of virus through the external nares or choanae. Studies have demonstrated that this occurs infrequently in chickens vaccinated by the drinking water route, which results in a large proportion of the vaccinated flock failing to develop protective immunity (47). Application of ILT vaccines by spray may result in adverse reactions as a result of insufficient attenuation of vaccine virus, deep penetration of the respiratory tract due to small droplet size of spray, or excessive dosage (12).

Differentiation of wild-type and modified-live vaccine viruses may be accomplished by restriction endonuclease cleavage of viral DNA and electrophoretic separation of DNA fragments. This method has been used extensively in epidemiological studies of field outbreaks to identify wild-type and vaccine

viruses (32). Other methods of differentiating ILTV strains, such as DNA hybridisation (37) and analysis of virulence for chicken embryos (25), have not been sufficiently evaluated. Serological differentiation of wild-type and vaccine virus exposures is not currently possible. Strains of ILTV are antigenically homogenous (based on VN, IF tests and cross-protection studies).

Eradication of ILTV from intensive poultry production sites appears to be highly feasible owing to the natural biological and ecological properties of the virus, as proposed by Bagust and Johnson (6). These properties include the high degree of host-specificity of the virus, the relative fragility of ILTV infectivity outside the chicken, and the antigenic homogeneity of the virus. Furthermore, the chicken is the primary host species as well as the reservoir host; wildlife reservoirs are believed either to be non-existent or of minor importance in ILTV ecology. Antigenic homogeneity of the virus allows use of a single ILTV vaccine to provide cross-protective immunity for all ILTV strains.

Backyard and fancier-show chicken flocks are likely reservoirs of ILTV. Thus, any eradication effort would require identification and inclusion of these flocks in eradication programmes (40).

Import/export concerns

Infectious laryngotracheitis is found world-wide, particularly in those countries with intensive poultry production. Importation of the virus into these endemic countries would not be a concern unless importation is from an area where particularly virulent strains of ILTV exist. In those countries or geographic regions that are free of ILTV, measures should be taken to prevent introduction of the virus. Most importantly, live chickens and pheasants should be excluded from importation, unless from flocks which are antibody-negative or from regions known to be free of ILTV, as these birds may be

carriers of the virus (birds may be actively infected, latently infected or mechanical carriers). For the same reason, ILTV-vaccinated birds should be excluded from importation. Importation of hatching eggs and hatchling chickens should not pose a risk as ILTV is not transmitted vertically. However, caution should be exercised in the importation of hatching eggs and hatchling chickens, as ILTV could be introduced via contaminated fomites, such as egg crates, especially as the virus has been demonstrated to survive for long periods in organic material. Imported poultry meat, bone meal and most poultry by-products do not pose a significant risk, as ILTV infection involves only respiratory tissues. Provided that meat-and-bone meal and other products are not contaminated with respiratory tissues, these products pose no hazard for introduction of ILTV.

Conclusion

While ILTV disease is comparatively easy to distinguish clinically and in the laboratory, localised in distribution and potentially excludable from most developed intensive poultry industry sites by sound biosecurity measures, eradication cannot be envisaged while vaccines based on modified-live ILTV strains are in use. Meanwhile, ILTV disease remains a constant threat through outbreaks originating from chickens on production sites which are reservoirs of ILTV infection. The authors hope that the emphasis accorded in this paper to understanding the strong links between the persistence of ILTVs and the movement of live chickens, can also assist in achieving more consistent control of ILTV in poultry industries generally. This will be required for trade compliance in the movement of poultry both regionally and internationally.

Laryngotrachéite infectieuse aviaire

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Résumé

L'herpèsvirus responsable de la laryngotrachéite infectieuse aviaire est à l'origine de foyers sporadiques de cette maladie respiratoire des volailles dans le monde entier. Trois voies de transmission sont possibles pour la maladie : par des volailles présentant une maladie aiguë des voies respiratoires supérieures, par des poules « porteuses » à infection latente qui excrètent le virus de la laryngotrachéite infectieuse (VLT) en cas de stress, et par des vecteurs mécaniques (tous objets inanimés ou personnel en contact avec des poulets

infectés). Le pouvoir infectieux du VLTI peut persister de quelques semaines à quelques mois dans la muqueuse trachéale ou dans les cadavres. La prophylaxie repose dès lors sur une biosécurité rigoureuse dans l'établissement. De plus, si les vaccins actuels (à virus vivant modifié) offrent une bonne protection, les souches vaccinales peuvent parfois provoquer des infections latentes, voire de nouveaux cas de laryngotrachéite infectieuse après transmission entre volailles. La répartition régionale des réservoirs d'infection compromet l'efficacité des mesures prophylactiques, lesquelles varient considérablement selon les élevages; cela entraîne fréquemment des cas sporadiques et inattendus de laryngotrachéite infectieuse dans certains élevages intensifs. Les précautions applicables aux déplacements de volailles de tous âges doivent par conséquent inclure une connaissance précise de la situation sanitaire des élevages d'origine et de destination.

Mots-clés

Biosécurité – Commerce international – Épidémiologie – Herpèsvirus aviaire 1 – Maladies aviaires – Quarantaine – Répartition régionale – Virus de la laryngotrachéite infectieuse aviaire.

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Resumen

El herpesvirus de la laringotraqueítis infecciosa aviar sigue causando en todo el mundo casos esporádicos de afecciones respiratorias en el pollo. La infección puede transmitirse a partir de tres fuentes: pollos con infección aguda del tracto respiratorio superior; aves 'portadoras' de una infección latente que excretan el virus en situaciones de estrés; y por último fómites de todo tipo (ya se trate de objetos o del personal en contacto con pollos infectados). El virus de esta enfermedad puede conservar su infectividad durante semanas o meses en el mucus traqueal y los cadáveres de pollos. Por tal motivo, para controlar la enfermedad es fundamental velar muy estrictamente por la seguridad biológica de las instalaciones. Además, aunque las actuales vacunas (con virus vivos modificados) ofrecen un buen nivel de protección, las cepas víricas empleadas pueden también provocar infecciones latentes, así como la aparición de la enfermedad si se produce transmisión entre ejemplares. La distribución regional de los reservorios de bandadas infectadas por el virus tiende a restar eficacia a las medidas de control – extremadamente dispares – que aplica el sector productivo, lo que a su vez provoca de vez en cuando brotes esporádicos e inesperados de laringotraqueítis entre las poblaciones aviares de explotaciones de carácter intensivo. Por ello, entre las precauciones adoptadas de cara al movimiento comercial de pollos de cualquier edad debe figurar un exacto conocimiento de la situación sanitaria de las bandadas de origen y destino con respecto a esta enfermedad.

Palabras clave

Comercio internacional – Cuarentena – Distribución regional – Enfermedades aviares – Epidemiología – Herpesvirus aviar 1 – Seguridad biológica – Virus de la laringotraqueítis infecciosa aviar.

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