LARYNGOTRACHEITIS

Slide Study Set #15

A Continuing Education Program Revised by

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INTRODUCTION

Laryngotracheitis (LT) is an acute viral respiratory disease primarily of chickens. Economic losses attributable to LT are increasingly important in many poultry producing areas throughout the United States and the world. In addition to chickens, pheasants and peafowl are susceptible to LT infection (7, 13, 20, 21).

CLINICAL SIGNS

Clinically, most flocks exhibit severe respiratory disease including difficulty in breathing and expectoration of blood from the trachea. Other flocks have only a mild respiratory disease and conjunctivitis (25). In some layer flocks there may be no change in egg production, while in other cases there may be a decrease in production of 5-15 % with no change in egg shell quality (7). The clinical presentation may vary due to the strain of the virus and other environmental and host factors (23).

Mortality varies greatly between flocks. In broilers, this mortality ranges from 0.7% to 50%. In pullets, the mortality ranges from 1.3% to 16%. In layers, the mortality associated with disease has ranged from 0% to 12%. The total mortality in broiler flocks maybe an underestimation because these flocks are often marketed during an outbreak. Daily mortality in pullet and layer flocks does not follow a pattern but daily mortality in unvaccinated broiler flocks characteristically doubles each day after onset of clinical signs (7).

LESIONS

Postmortem lesions are primarily confined to the trachea. Occasionally, pneumonitis and airsacculitis are seen. The most common postmortem lesions are hemorrhage and/or caseous material in the trachea, however some flocks do not show the classical form of the disease. In these flocks, conjunctivitis, sinusitis and mucoid tracheitis may be the only lesions (13, 24). Although experimentally, pulmonary and airsac lesions were consistent findings in birds affected by the aerosol route (27). Secondary bacterial infections are seldom seen in conjunction with LT. Although, in broilers which break with LT at 3 to 4 weeks of age and stay in the field for additional 3 to 4 weeks prior to processing severe *E.coli* airsaccultitis has been seen. Concurrent viral infections are also uncommon (7).

PATHOGENESIS AND EPIDEMIOLOGY

Transmission between flocks has primarily been associated with their geographical proximity and a breakdown in biosecurity. Movement of personnel, improper dead bird and manure disposal and exchanging of farm equipment have all been associated with LT outbreaks (7).

Laryngotracheitis is caused by an Alphaherpes virus. Carriers are produced by either previous exposure to field virus or vaccine virus (5, 15, 17, 32). Inoculated birds will intermittently shed virus between 7 and 20 weeks postinoculation (6, 7, 14) . The main site of latency for ILT virus has been shown to be the trigeminal ganglion (5, 32) and the trachea (5, 15, 17). Reactivation of latent virus has been suggested as the cause for clinical disease in some flocks (15).

Research has also shown through backpassage that the chicken embryo-origin vaccine virus, not the tissue culture- origin vaccine virus, may revert to the more pathogenic parent strain. A modified-live CEO product was passaged 20 times in specific pathogen free chickens. After 10 chicken passages, there was an increase in virulence consisting of an increase in mortality and an increase in the severity and duration of the disease (11).

Strains of LT viruses have been defined by restriction enzyme analysis of viral DNA (4, 10, 20, 21) and by DNA:DNA hybridization using a cloned DNA marker (23). Recently, it has been shown through restriction endonuclease analysis of both vaccine and field isolates, that outbreaks of LT may be related to vaccines or vaccine-like viruses (10). Other researchers have found strains of LT different from the chicken-embryo-origin and tissue-culture-origin vaccines (4, 20, 21). Current restriction endonuclease analysis may not be sensitive enough to differentiate between field isolates and vaccine strains (20).

DNA:DNA hybridization with a cloned marker has been used for the differentiation between virulent and avirulent strains of ILT. An ILT DNA clone was isolated which allows for pathotyping of strains by hybridization (23).

DIAGNOSIS

Historically, diagnosis has been based upon postmortem lesions, histopathology, virus isolation, or immunofluorescent antibody and virus neutralization test. Additional procedures which are used for the diagnosis of ILT include A non-isotopically labeled DNA probe (19, 22) immunoperoxidase (12), ELISA (34), electronmicroscopy (16, 31), and PCR (29, 33).

Histopathology

Microscopic lesions of the trachea include degeneration and necrosis of epithelial cells with syncitia containing the intranuclear inclusion bodies, usually found in the tracheal lumen. The inclusion bodies may be difficult to find 5 days post infection. At this time, the trachea is lined by hyperplastic nonciliated epithelial cells. Lesions can also be seen in the bronchi, lungs and airsacs. Pneumonia may be present in the ventral lung and surrounding primary bronchi. Fibrin, heterophils and syncitia containing intranuclear inclusion bodies may be seen in the tertiary bronchi. Airsac lesions, in experimentally infected birds, may include hyperplasia of the epithelium and syncitia with intranuclear inclusion bodies and fibrosis (8, 26).

Virus Isolation

The best samples for isolation of LT are tracheal exudate, tracheal tissues or lung tissues. Isolation for LT virus is by the chorioallantoic membrane route (CAM) route of inoculation in 9-to-12-days-old chicken embryos (30). Plaques are produced on the CAM and the size of the embryo may be reduced. Susceptible chickens may also be inoculated by tracheal, infraorbital sinus or aerosol routes. Clinical respiratory disease and postmortem lesions may vary in severity based on the viral isolate, age of bird and route of inoculation (30). Cell culture in chicken embryo liver and chicken embryo kidney monolayer cultures may also be used for isolation. Cytopathic changes include the development of multinucleated polykaryocytes or giant cells with a few cells having intranuclear inclusion bodies (30).

Fluorescent Antibody

A direct immunofluorescent antibody test may be used as an alternative or supplemental diagnostic test. This test has been found to be as effective as histopathology for the diagnosis of LT in chickens (9).

Virus Neutralization

Virus neutralization can be used for the detection of virus or antibody (30). Due to the cost and time needed for completion of the test, it is not readily used as a diagnostic test but rather as a research tool.

Additional Diagnostic Tests

A DNA probe, dot blot hybridization assay has been developed for detection of LT virus. Virus isolation is useful in the beginning of the infection where there are low numbers of virus but, later in the infection when the virus is inactivated or becomes latent the DNA probe is more sensitive (19, 22).

A monoclonal antibody-based immunoperoxidase (IP) procedure was compared with an indirect immunofluorescent antibody procedure, histopathology and virus isolation. The IP procedure had a sensitivity and specificity of 72 and 93% respectively when compared to virus isolation (12). Additional studies have indicated that a monoclonal-based IP test was more sensitive but less specific than histopathology, PCR and hybridization. In comparison to the indirect immunofluorescent antibody test IP was more sensitive and had similar specificities (1).

An antigen-capture-enzyme-liked immunosorbent assay (ELISA) has been developed which was as sensitive as virus isolation and more sensitive than the flourescent antibody test. It was able to differentiate chickens infected with LT virus and those that were uninfected (34).

Electron microscopic examination may be used as a diagnostic tool for the detection of LT viral particles (16,31,33). It was used for rapid diagnosis after primary isolation of ILT virus using chicken embryo liver cells (16). It has also been demonstrated to be a sensitive diagnostic test using scrapings from the trachea in suspected ILT infected birds (31). In another study comparing electron microscopy, virus isolation and polymerase chain reaction, it was found that electron microscopy was the least sensitive method for detection of ILT virus (33).

PCR in the comparison study with electron microscopy and virus isolation was found to be the most sensitive test for detection of ILT virus (33). A combination test of the polymerase chain reaction and a DNA colourimetric assay was used to detect DNA from ILT virus. This assay was sensitive and specific (29).

Serology

Serology is not a primary diagnostic tool for LT. Immunity to LT viral infection is considered to be mainly related to cellular immunity rather than humoral immunity (18,28). This conclusion was based on studies where birds were bursectomized surgically at day of age, subsequently treated with cyclophosphamide therapy and then vaccinated for LT. Birds challenged with LT virus did not produce antibody but were immune (28).

Some authors suggest that the ELISA is a rapid and accurate test to determine the immune status of a vaccinated or infected flock (35). A commercially available ELISA was tested as a potential screening test for antibody to LT viral infection. Since humoral antibody production is not the primary immunological response to infection the authors suggest that serological monitoring will not differentiate infected, asymptomatic or uninfected flocks (24). To diagnose LT at a subclinical level, it may be best to use a labeled avidin-biotin-based ELISA, a monoclonal-antibody-based ELISA and a cloned DNA probe. These may be more sensitive and specific than the commercially available ELISA (24). The ELISA was found to detect vaccine-induced antibody but could not reliably identify old flocks which were previously vaccinated with the tissue culture vaccine (24).

VN titers to LT have been used as an indication of flock protection. The flock VN GMT correlates with protection against challenge but individual titers do not correlate with challenge (3).

The following serologic tests have been used in conjunction with other laboratory tests to evaluate immunity or exposure to LT: agar gel immunodiffusion test (AGID) (31), serum neutralization test, direct and indirect immunofluorescent antibody test, and enzyme-linked immunosorbent assay. AGID has been found to be less sensitive than the ELISA, FA or SN test (2).

CONTROL AND PREVENTION

Control and prevention is through vaccination with either chicken embryo vaccines or tissue culture vaccines. Although eyedrop administration is recommended by the manufacturer, the poultry industry often administers chicken embryo products by spray or water. Commercial layers and breeders are usually vaccinated twice prior to the onset of lay. These flocks are usually vaccinated at 7 weeks by eyedrop and again at 15 weeks by either eyedrop, water or spray. In some areas, broiler breeders are vaccinated only once at 8-12 weeks of age. Broilers are usually not vaccinated unless they are in the vicinity of an outbreak or a previous outbreak has occurred on the farm. When this occurs, they are then vaccinated at 10-12 days of age in the water (6).

Vaccination may also be used in the face of an outbreak. Both water and spray vaccination has been used with success in reducing the spread of the disease within a flock.

DIFFERENTIAL DIAGNOSIS

Differential diagnosis for the mild form of the disease must include respiratory diseases such as avian influenza, infectious bronchitis, Newcastle disease and mycoplasmosis. The more severe form of the disease should be differentiated from the diptheritic form of avian pox (13).

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Slides

Slide 1: Chicken having difficulty breathing due to infection with laryngotracheitis.

Slides 2 and 3: Chicken with conjunctivitis related to laryngotracheitis infection.

Slide 4, 5, 6 : Gross pathologic tracheal lesions associated with various stages of laryngotracheitis infection.

Slide 4: Hemorrhage in the trachea
Slide 5: Fibrinohemorrhagic tracheitis
Slide 6: Caseous plug in the trachea

Slide 7: Low power (10X) micrograph of the trachea. Of note is the pronounced desquamation, mucosal inflammation and synctia (multinucleate cell) formation visible.

Slide 8: High power (40X) micrograph of the tracheal lumen debris from a case of laryngotracheitis. Note the numerous intranuclear inclusion bodies in sloughed epithelial cells and synctia formation.

Slide 9: High power (40X) micrograph of the conjunctiva of a bird infected with laryngotracheitis. Note charcteristic epithelial intranuclear inclusions.

Slide 10: Plaques on the chorioallantoic membrane after inoculation of chicken embryos with laryngotracheitis virus.

Slide 11: Low power micrograph (10X) of the chorioallantoic membrane (CAM) showing diffuse CAM thickening and plaque formation following innoculation with laryngotracheitis virus.

Slide 12: High power micrograph (40X) of the chorioallantoic membrane (CAM) showing characteristic inclusion bodies in the CAM following the inoculation of laryngotacheitis virus.

Slide 13: Low power magnification (4X) of a relatively high MOI (1-2) infection of chicken embryo liver (CEL) cells with the NVSL challenge strain of ILTV virus 20 hours post-infection. One corner of the slide monolayer is still visible. In the center are several rounded balls of infected cells which are characteristic of an ILTV infection.

Slide 14 and 15: Low magnification (4X) micrograph 48 hours after infection showing unstained monolayers of CEL cells containing individual ILTV plaques.
Four plaques are visible in slide 14 and one plaque in slide 15. The virus will make holes in the monolayer, within which are clumps of dead cells. This is very typical of ILTV cytopathic effect.

Slides 16 and 17: Low magnification (10X) of infected CEL monolayers that have been stained with crystal violet. They show two important characteristics of ILTV plaques. The three plaques in slide 16 demonstrate how ILTV plaques differ from holes in a monolayer. The edges of the plaque are very sharp and clearly defined and usually contain a clump of dead cells. These characteristics are also clearly seen in the one plaque in Slide 17. In addition, this slide shows how ILTV does not infect chicken embryo fibroblasts since they have filled in the hole in the CEL monolayer made by the virus.

Slide 18: BamHI digest of 9 strains of ILTV. Lane 1 – USDA challenge virus, Lane 2 – tissue culture adapted vaccine strain, Lane 3 – chicken embryo propagated vaccine strain, Lanes 4 –9 – field isolates. The chicken embryo propagated vaccine strain can be differentiated from the other two control strains, but not from any of the field isolates. Strain 481 is in lane 4.

Slide 19: RFLP analysis using the 4 base cutting enzyme Sau3AI. As in slide 18 the differences are minimal. However, a unique 235 bp fragment is observed in field isolate 481 (Lane 5) which is not found in a typical chicken embryo propagated vaccine strain (lane 4).

Slide 20: Immunflorescent antibody of ILTV plaques of infected CEL monlayers. The three plaques were incubated with polyclonal rabbit antiserum to glycoprotein B and then reacted with FITC conjugate anti-rabbit antibody. The intensity of the fluorescence usually is not uniform around the margin of the plaque.

Slides 21 and 22: Low power micrograph (20X) and high power magnification (40X) of fluorescent antibody conjugate-stained cells. Chicken embryo kidney cells were infected with the second passage of the ILTV challenge virus LT 83-2 from NVSL. The cells were fixed 48 hours post-infection and stained with ILT direct fluorescent conjugate propared against LT 83-2 at NVSL.

Slides 23, 24, and 25: Negative stained ILTV taken by electron microscopy. The stain was 2% phosphotungstic acid at pH 7.0. The magnification bar on each slide is 100nm. The total magnification for slides 23 and 24 is x180,000 and for slide 25 is x138,000. Slides 23 and 24 depict an icosahedral tubular nucleocapsid enclosed within an envelope. Slide 25 depicts 3 virions, 2 in envelopes and one that is only the viral nucleocapsid. The dark stained nucleocapsid are empty particles devoid of DNA.

Slide 26: High power micrograph (X45) of positive Immunoperoxidase staining of chicken trachea infected with ILTV 4 days post-infection. (Red filter)

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