

CEVA HANDBOOK OF POULTRY DISEASES

9 LARYNGOTRACHEITIS

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LARYNGOTRACHEITIS

DEFINITION

Laryngotracheitis (LT) is a viral infection found in hens, pheasants and peacocks, characterized by catarrhal haemorrhagic or fibrinous inflammation of the respiratory tract. It is manifested in laryngotracheal and conjunctival form.

HISTORY

The first report of LT dates back to 1925, when the disease was described in the USA as tracheo-laryngitis (May & Tittsler, 1925). Over the next years, cases were reported in many countries with intensive poultry production in Europe, South and North America, Asia and Australia, where it became a serious concern (Hidalgo, 2003). Some areas have developed endemic LT problems, especially farms with mixed-age flocks and those in vicinity to backyard birds. In

the first years after the disease was detected, it was variously referred to as infectious laryngotracheitis, avian diphtheria, and infectious bronchitis. The viral etiology was confirmed by Beaudette (1930). Seifried (1931) observed that lesions affected the larynx and the trachea and defined the disease as infectious laryngotracheitis. LT is the first important viral avian disease for which a vaccine has been developed (Brandly & Bushnell, 1934).

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

The virus causing avian laryngotracheitis is taxonomically determined as *Gallid herpesvirus 1* (GaHV-1) (Roizman, 1996). GaHV-1 is a member of the Herpesviridae family, subfamily Alphaherpesvirinae, genus *Iltovirus*. It possesses the specific family morphological features: envelope, 162 capsomeres, and a size of 125 to 200 nm (Zarkov, 2003).

On the basis of virus neutralization, immunofluorescence and other tests it is generally accepted that GaHV-1 strains are antigenically homogenous, but show variations with respect to virulence against the host (Bagust et al., 2000). Naturally-occurring GaHV-1 strains vary from high-virulence, provoking substantial morbidity and mortality



rates, to low-virulence, producing mild or undetectable infection. The nucleic acid is DNA. Viral glycoproteins, similarly to other herpesviruses, are the major immunogens responsible for stimulation of humoral and cell-mediated immune responses (Hidalgo, 2003).

The virus of LT is relatively resistant. In a dead bird's trachea, it survives 24-48 h at 37°C, at room temperature for up to several days (5-6), in faeces for 3 days; and in deep litter it can survive for anywhere from 3 to 20 days. In frozen meat, it may survive 1-2 years, and in lyophilized state more than 13 years. Heat, light, putrefaction and common sanitizers (formalin, sodium hydroxide, cresol etc.) kill it quickly.

The replication of the virus occurs in the epithelium of upper respiratory tract mucosa (larynx, trachea, nasal and oral cavity, conjunctivae). Viral DNA transcription and replication takes place in the nucleus (Prideaux et al., 1992; Guo et al., 1993).

DNA nucleocapsids acquire an envelope migrating through the inner lamella of nuclear membrane. Next, enveloped particles migrate through the endoplasmic reticulum and accumulate in cytoplasmic vacuoles (Guo et al., 1993). The release of enveloped viruses occurs by cell lysis or lysis of vacuole membrane and exocytosis. The presence of viruses at predilection sites results in catarrhal haemorrhagic inflammation of the mucosa with inflammatory intercellular oedema (*status cribrus*) resulting in desquamation catarrh. Frequently, a secondary infection (*E. coli*, *St. aureus*, *M. gallisepticum* etc.) is observed, generally leading to the appearance of fibrinous caseous exudate. The infective agent usually persists in the tracheal tissue and secretions for 6 to 8 days, during which period it is shed in the environment (Bagust et al., 1986). There is no evidence supporting the presence of a viraemic stage of infection.

EPIDEMIOLOGY

Chickens are the most commonly affected birds.

Cases of LT have also been reported in pheasants (Crawshaw & Boycott,

1982), lesions induced in turkeys and LT virus isolation from peacock tracheae (Winterfield & So, 1968). Other hosts or reservoirs of infections are not known (Bagust et al., 2000). LT can affect birds of all ages, although the most pronounced clinical signs are observed in adult birds (Guy & Garcia, 2008). Clinically, the disease is most commonly observed between the ages of 5 and 12 months (Girginov & Iliev, 1984).

Both diseased birds and those in remission may act as sources of further infections. Birds which recover from LT may act as reservoir hosts for a further 12–24 months. Backyard fowl are probably reservoir hosts as well (Bagust et al., 2000). The infection may remain latent and the main site of latency is the trigeminal ganglion (Bagust, 1986). Re-excretion of GaHV-1 from latently infected chickens, resulting from the stress of moving the flock into a new facility before egg production starts, has also been reported (Hughes et al., 1989). The infection may be introduced to the farm via contaminated water, feeds, equipment, personnel etc., which are passively contaminated by excreta of clinically ill birds. Evidence for the role of ventilation systems in poultry barns and the wind as vectors of LT transmission has been published (Johnson et al.,

2005). Other vectors spreading the infection among the premises within a farm are dogs, rats and even birds (crows) (Mallinson et al., 1981). The darkling beetle (*Alphitobius diaperinus*) is also believed to spread GaHV-1 (Ou et al., 2008).

Poor hygiene conditions (over-population, extreme temperature variations, increased humidity, inadequate ventilation), unbalanced diet, especially during the peak egg production and stress have also been singled out as causes predisposing to infection with LT.

The infection is transmitted mainly via the aerogenic and alimentary routes. The intraconjunctival route is also possible. No evidence exists for viral transmission via eggs or eggshells of infected birds (Bagust et al., 2000). Hatchlings are virus-free. The disease is enzootic. A certain stationary character is observed, which could be attributed to long-term virus carriership in recovered birds and the relatively high resistance of the etiological agent. An important factor in this respect is the previously discussed possibility for latent infection allowing periodical reactivation and shedding of the virus (Bagust et al., 2000). In very recently reported LT outbreaks, the endemic character of the disease in some regions of Australia has been outlined (Blacker et al., 2011).

CLINICAL SIGNS AND PATHOLOGY

The incubation period of naturally transmitted infection is usually 6–12 days, whereas in experimental infections it depends on the challenge route. After intratracheal inoculation or aerogenic exposure,

clinical signs appear after a short incubation period of 2–5 days (Benton et al., 1958; Jordan, 1963). Clinically, LT occurs in laryngotracheal and conjunctival forms.

LARYNGOTRACHEAL FORM

May be either mild, with very low mortality rates (0.1% - 2.0%), or severe, characterized by high

morbidity (90–100%), and death rates of between 5% and 70%, with an average of 10–20%..



Fig.1

Clinically, the laryngotracheal form is manifested by bouts of dyspnea, difficult breathing and rales. Usually, the head and the neck are strongly extended forward and upward when inhaling. Often, fresh or dried serous haemorrhagic discharge can be found around the nostrils.



Fig.2

In the initial stage, the laryngotracheal mucosa is hyperaemic, diffusely haemorrhagic, and the lumen contains various amount of catarrhal haemorrhagic exudate.



Fig.3

Sometimes, only blood coagula are found inside the tracheal lumen.



Fig.4

In more prolonged clinical course of the disease, the exudate in the larynx and the trachea becomes catarrhal haemorrhagic, coagulates and forms casts.

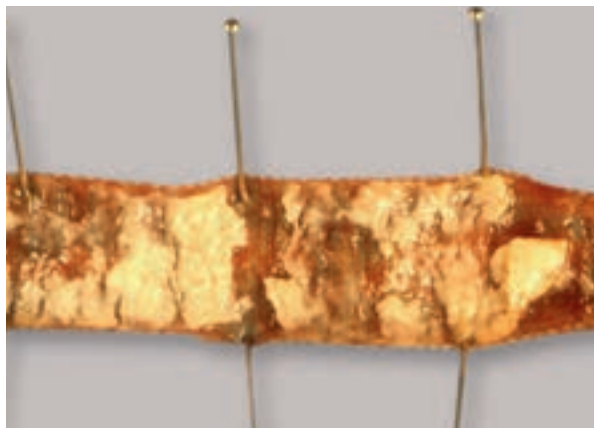


Fig.5

In more advanced stages, coagulated exudate becomes fibrous, caseous and may obstruct the laryngeal and tracheal lumens, either partially or completely..



Fig.6

In some older cases, a part of inflammation exudate is removed by expectoration, leaving visible areas with severely thinned and transparent tracheal wall (arrows).

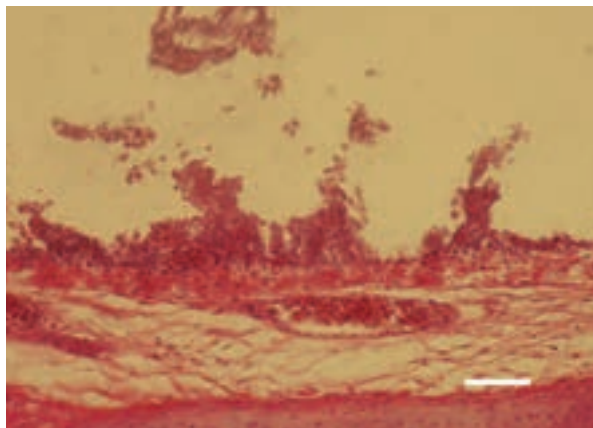


Fig.7

Trachea, a transverse cross-section. Severe haemorrhagic desquamative inflammation of the mucous coat. Erosions, ulcers and an extremely thinned mucous layer. H/E, Bar = 50 μ m.

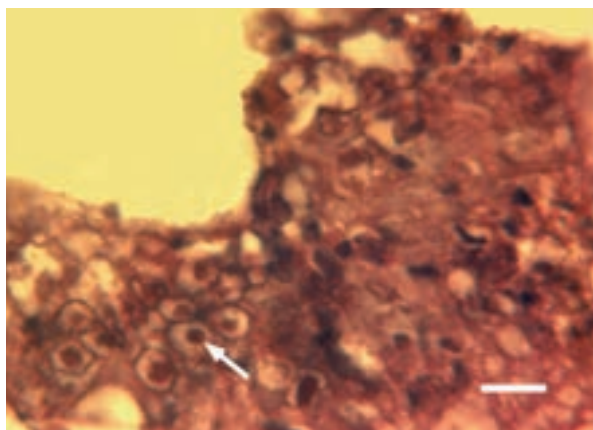


Fig.8

The detection of eosinophilic intranuclear inclusion bodies (arrow), surrounded by a light halo in the mucous coat epithelium is of essential diagnostic value. This is possible in the initial stage of the disease, prior to the occurrence of desquamative lesions. H/E, Bar = 10 μ m.

Conjunctival form



Fig.9

In the conjunctival form of LT, ocular and nasal discharge and, most commonly, catarrhal or catarrhal purulent conjunctivitis are observed.



Fig.10

After complicated infections, oedema of infra-orbital sinuses may be present.

DIAGNOSIS

A tentative diagnosis can be made on the basis of disease's history, as well as the clinical and morphological signs, but laboratory confirmation is essential. LT can be confirmed by one or several laboratory tests, including detection of intranuclear inclusion bodies, isolation and identification of the etiological agent, serological tests (VN, ELISA) or detection of GaHV-1 DNA (Guy & Garcia, 2008).

Histopathological investigation

Histopathological investigation is a standard, simple and rapid method of confirming the presence of LT. The specific lesions are inflammatory oedema, haemorrhages, inflammatory cell infiltration and necrosis, while pathognomonic signs include intranuclear eosinophilic inclusion bodies in the respiratory and conjunctival epithelium. When collecting samples for histopathology, it should be borne in mind that active viral replication occurs during the first 5-6 days after infection in the epithelium of affected mucosae.

The consequences include macrobiotic lesions and

desquamation catarrh with initial regeneration. The appearance of inclusion bodies is therefore transient, and if samples are collected at a later stage they may not be detected (Bagust et al., 2000). Inclusion bodies may be observed after Giemsa or haematoxylin/eosin staining of paraffin-embedded trachea cross sections. Longitudinal tracheal sections allow for better observation of inclusion bodies. Some more rapid techniques for processing tissue tracheal sections (taking less than 3 hours) have also been identified (Bagust & Guy, 1997).

Virus detection

Appropriate samples are respiratory discharge, tracheal and lung tissue. The samples should be transported in a manner which takes into account the lability of the virus. A good option is cooling in ice and transportation within 48-60h. It should also be remembered that the virus is inactivated at minus 10°C (Zarkov, 2003).

Perhaps the fastest method for detection of viral GaHV-1 particles is electron microscopy of negatively contrasted tracheal particles.

Herpesvirus particles may be detected within an hour of the sample arriving at the lab. In such cases diagnosis is based on visualization and identification of herpesvirus particles. The success of the method depends on the number of viral particles in a given sample (Hughes & Jones, 1988).

The virus can be relatively easily detected by agar gel immunodiffusion (AGID) using hyperimmune serum against GaHV-1 (Jordan, 1962). For this test, macerated laryngeal or tracheal tissue from ill birds may be used.

The LT virus can be isolated by inoculation of the supernatant obtained from tracheal exudate or homogenate, treated with antibiotics, in the chorioallantoic membrane of 10-12-day-old embryonated chicken eggs. Three days later, nodules 1–3 mm in size appear along the blood vessels of the chorioallantoic membrane. Samples for cultivation should be obtained as soon as possible after the manifestation of clinical signs, as isolation could fail if attempted after 6–7 days (Bagust & Guy, 1997). In addition to chick embryos, the virus may be cultivated in cell cultures where it exhibits cytopathic effects, including formation of large syncytia of 80–100 nuclei (Zarkov, 2003).

For LT virus identification, the

immunofluorescence (IF) or immunoperoxidase (IP) tests, virus neutralization (VN) reaction, ELISA, DNA hybridization techniques and PCR may be used. The IF test confirms the presence of virus through the appearance of perinuclear cytoplasmic fluorescence. The use of monoclonal antibodies is an advantage of the IP test (York & K.J. Fahey, 1988). Protocols for detection of LT viral DNA by PCR have been proposed by several research teams (Williams et al., 1994; Abbas et al., 1996; Chang et al. 1997; Alexander et al., 1998).

The impossibility of distinguishing between field and vaccinal LT strains caused problems in the detection of GaHV-1 for a long time. A solution was finally found in the form of a method allowing for rapid differentiation of vaccinal and field isolates by restriction fragment length polymorphism (RFLP) of PCR products, proposed by Chang et al. (1997). This method, in the view of Bagust et al. (2000) was highly promising in that it represented a significant improvement in our understanding of LT virus epidemiology and evolution. At present, PCR-RFLP is successfully used for genotype characterization and classification of LT viral isolates (Oldoni & Garcia, 2007; Blacker et al., 2011).

Antibody detection

Various techniques are used to detect antibodies acting against GaHV-1 in chicken serum, including

ELISA, AGID, VN and indirect immunofluorescence (IIF) (Guy & Garcia, 2008)..

DIFFERENTIAL DIAGNOSIS

LT should be differentiated from other poultry disease manifested with respiratory signs and lesions: infectious bronchitis, infectious coryza, swollen head syndrome,

fowlpox (wet pox), chronic respiratory disease and avian influenza.

PREVENTION AND CONTROL OF LT

The prevention and control of LT requires a combination of non-specific (biosecurity) and specific (vaccinations) measures. These measures are applied in problematic stock layer and broiler breeder farms. Practically, these measures are applied only in endemic regions with intensive poultry production. Vaccination is not commonly practiced in broiler fattening farms due to the short production cycle.

Vaccination

The use of vaccines is recommended only in endemic regions, as vaccination may produce latent infection carriers. Approval of vaccines and application protocols must be sought from an authorized agency (Guy & Garcia, 2008).

By now, the vaccines used for prophylactic vaccination are mostly modified live LT vaccines. They are prepared by multiplication of laboratory attenuated vaccinal strains or natural avirulent strains

in cell culture or chick embryos. When correctly used, modified live LT vaccines are highly effective and can protect susceptible birds, if a penetration of the virus occurs at a later stage (Hitchner, 1975).

Vaccines are applied orally (in drinking water), by spraying or by eye instillation. After aerosol vaccination, side effects may occur as a result of insufficiently attenuated vaccinal viruses, penetration in the lower respiratory tract due to small droplets size, or overdosage (Clark et al, 1980). Successful vaccination via drinking water depends on the contact of vaccinal water with the nasal epithelium, resulting in aspiration of the virus via the nostrils or choanae. There are, however, reported cases where flocks vaccinated using this method have failed to develop full protective immunity (Robertson & Egerton, 1981).

Vaccines for LT control have been developed based on recombinant DNA technologies. Recombinant live LT vaccines were created by alteration or omission of viral genes which code for the virulence factors. In this way, recombinant vaccines are able to induce a protective immunity without provoking disease (Guy & Garcia, 2008). Vector technology, using fowlpox or HVT as carrier of one or several

immunogenic epitopes from LT virus, is yielding promising results. Another approach for induction of protective immunity is genetic immunization. It is based on the fact that plasmid DNA is non-infective and does not replicate. Moreover, plasmid DNA is stable and can be stored in conditions that would otherwise destroy the virus. Another important advantage is the potential for application by various routes, including in ovo administration. The practical and economical impact of these vaccines for the poultry industry is yet to be confirmed (Hidalgo, 2003).

Eradication

It is anticipated that the LT virus eradication will be easier in the future. The development of genetically engineered LT vaccines induces a protective immunity without latent infection in carrier birds (Bagust & Johnson, 1995).

Biosecurity

Non-specific prevention and control includes those biosecurity procedures generally applied in poultry industry management.

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