

Outbreak of Infectious Laryngotracheitis Following Vaccination in Pullet Flock

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Summary

An outbreak of infectious laryngotracheitis (ILT) in a large flock following vaccination against ILT is described. Laryngotracheal samples were obtained from some of infected pullets. Isolation and characterization of agent virus were carried out on chorioallantoic membrane (CAM) of embryonated specific pathogen free (SPF). Several pocks on CAM were observed after five days of incubation. The isolated virus was neutralized by mono-specific antiserum against vaccinal ILT virus. For evaluation of pathogenicity of the isolated virus twenty 8-week-old SPF chicks were eye drop inoculated with 10^3 and 10^4 EID₅₀% virus respectively and observed upto 7 days. Only two chickens in group 2 were shown moderate signs including conjunctivitis and swelling of lower eyelid. Histopathologic features in tracheal of both infected pullets and experimental SPF chicks were epithelial hyperplasia, multinuclear giant cells (syncytia) with eosinophilic intranuclear inclusion bodies and infiltration of inflammatory cells, which was milder in latter. These findings indicate that the isolated virus was similar to vaccine strain. Our study suggests that under improper ILT vaccine administration and generally bad management practice conditions, the ILT may occur following vaccination.

Key words: infectious laryngotracheitis, vaccination, pullet, outbreak

Introduction

ILT is an acute respiratory tract infection of chickens caused by Gallid herpes virus type 1 that characterized by signs of respiratory depression, gasping and expectoration of bloody exudate. The disease may result in severe production losses due to mortality and reduce egg production (Tripathy 1998, Wernery 2000). The disease may appear in three forms, preacute, subacute and mild or chronic. Chicken of all ages are susceptible, although the disease may be common in those aged 4-18 months (Jordan 1993).

Because of economic losses attributable to ILT, in endemic areas vaccination of susceptible chicken population with low-virulence ILT strain or attenuated [modified-live (ML)] viruses is recommended (OIE 1996). ILT viruses are attenuated by passage in cell cultures, embryonated eggs or by feather follicle passage in chickens. The immunity can be induced by vaccination of chickens via intranasal instillation, infraorbital sinuses (eye drop), coarse spray, cloacal scarification, drinking water and feather follicle. Despite efforts to prevent ILT through use of ML vaccine viruses, cases of vaccinated flocks that suffered outbreaks of clinical ILT were cited and it was indicated that vaccinated chickens could become infected with ILT viruses (Leib *et al* 1986, Guy *et al* 1991). ILT virus has been reported to spread from vaccinated birds to unvaccinated contact birds (Fulton *et al* 2000). Two hypotheses were made to confront the problem, including spread of vaccine virus to unvaccinates and potential reversion of ML vaccine viruses to parental-type virulence. However, ILT could also have been caused by insufficient attenuation of ML vaccine viruses or potentiation of normally innocuous ML vaccine viruses by undefined infectious, nutritional, or management factors (Guy *et al* 1990). In this paper we examine the debated question whether unexpected cases of ILT occurred in chickens shortly after vaccination.

Materials & Methods

Case history. The layer flock (Tehran province) was composed of 50,000 chickens. They were raised on five widely separate farms. An outbreak of a respiratory disease was detected on July 2000, when, ML ILT vaccine was administrated at 8-week-old by eye dropping method. The affected chickens had shown clinical signs including

spasm, coughing, oral and nasal discharge, haemorrhagic conjunctivitis, swelling of eyelids and infraorbital sinuses within 4-5 days of postvaccination. The mortality rate was about 3.1% at the end of disease's period.

Virus isolation. After removed tracheas with less contamination, exudate and epithelial cells were scraped from the tracheas diluted approximately 1/10 in nutrient broth containing 100 IU/ml penicillin and 100µg/ml streptomycin, then agitated vigorously. To remove debris the suspension centrifugated at 2000 rpm in 4°C. 0.2ml of supernatant was inoculated on to the dropped CAM of five 11-day-old SPF chicken eggs incubated at 37°C and 65% relative humidity up to 7 days. They were candled daily and the CAMs of dead embryos or of those surviving for 7 days, which had typical pocks harvested aseptically. The CAMs homogenized with ultra-turrax in 1/5 PBS of pH7.2 by 2×1min homogenization cycle of 7000 rpm. The suspension was frozen and thawed twice, then clarified at 5000rpm for 30min at 4°C. The supernatant fluid was harvested, titered and stored at -70°C until it was used.

Histopathology. Segments of trachea of infected chickens, approximately 3cm caudal to the larynx, were placed in 10% buffered neutral formalin and processed routinely for histopathology. Sections were cut at 5 microns and stained with haematoxylin & eosin. The severity of the lesions and the presence or absence of intranuclear inclusion bodies was noted.

Virus neutralization (VN) test. The α -procedure (constant-serum, diluted-virus) was used for serological identification of the isolated virus. 10-fold dilution of virus was added to undiluted mono-specific antiserum against ML ILT virus. The mixtures were incubated at room temperature for 1h to allow any neutralization to occur. The virus/serum mixtures were incubated onto the dropped CAMs of 11-day-old fertile SPF chicken eggs, using 6 eggs per dilution. Eggs were sealed and incubated at 37°C for 7 days. The end point was recorded, at the highest dilution of the virus, no pocks were present on the CAMs.

Experimental chickens. SPF fertile eggs were purchased from Lohmann Company (Cuxhaven, Germany), incubated in setter under sterile condition. After hatched, they were housed in separate cages in an isolation room with controlled traffic flow until

chickens were 8 weeks of age. Food and water were available *ad libitum*. Twenty chickens were divided into groups 1 and 2, 10^3 and 10^4 EID₅₀% of isolated virus inoculated by eye dropping method respectively and observed daily up to 7 days after inoculation.

Results

Of the 50,000 8-week-old chickens vaccinated with ML ILT, 1537 (3.074%) died. The postmortem lesions including mucoïd exudate with blood in trachea, yellowish caseous diphtheric membranes adherent to the larynx and upper respiratory tracheal mucosa were present in the birds. The histological lesions consisted of epithelial hyperplasia giving rise to multinucleated giant cells (syncytia) with eosinophilic intranuclear inclusion bodies and an invasion of inflammatory cells, desquamation of necrotic epithelial cells, and small areas of haemorrhage found in the lamina propria were demonstrated in infected chickens (Figure1).

Figure 1. *Typical appearance of the lesions of ILT virus including confluent pocks and edema in CAM after 6 days post incubation*

Isolation of agent virus was carried out by inoculation of tracheal samples on CAM and incubation at 37°C. Numerous pocks were formed on CAM (Figure2). Moreover,

the isolated virus was neutralized by monospecific antiserum. These findings indicate the isolation of a herpesvirus.

Figure 2. *Histopathological smear of infected trachea with ILT virus consists of multinucleated giant cells with eosinophilic intranuclear inclusion bodies, areas of hemorrhage and desquamation of necrotic epithelial cells*

No clinical signs were present in the SPF birds inoculated with 10^3 of isolated virus in group 1. Therefore, necropsy lesions (diphtheric and caseous necrotic plaques in trachea and larynx) were observed in two birds in group 2 can confidently be attributed to ILT infection. Their histological results were consistent with postmortem findings indicating ILT infection including mild infiltration of inflammatory cells and syncytia in a few areas.

Discussion

This study describes ILT infection in a layer flock following vaccination with a ML strain of ILT virus. The disease occurred while the chickens were vaccinated, under bad management practice condition, against ILT. Although a general ILT vaccination program that include all layer flocks, is applied in endemic areas, the program often

has not been effective. Problems may occur when vaccine is administered incorrectly and/or chick's immune system cells is affected by infection bursal disease, reticuloendotheliosis, chicken infectious anaemia, mycotoxicosis, and synchronized with other respiratory infections (Jordan 1993). So, vaccination fails to provide immunity to most birds in a flock, and biosecurity measures fail to prevent spread of vaccine viruses to unvaccinated flocks (Guy *et al* 1991, Fulton *et al* 2000). Factors influencing protection after vaccination with ML viruses include the age and health of the host, the route of vaccination, the tropism and the dose of the vaccine viruses (Jordan 1993). It is possible that lateral spread of virus was responsible for some of the infection seen (Bagust 1986). The present study clearly demonstrates that the isolated virus from recent case is similar to vaccine strain, which is used in Iran. Field isolates possessed greater virulence than vaccine virus, based on severity and duration of clinical illness, and tracheal lesions. Chickens may be exposed to ML ILT vaccine viruses through vaccination contact with previously vaccinated or otherwise exposed chickens, or mechanical transmission of these viruses from vaccinated flocks. The latter possibility seems to be the most probable source of the majority of isolate studied in this investigation. Previous report (Ebrahimi *et al* 2000) indicated that the ML ILT vaccine virus produced moderate tracheal mucosal damage in a few birds per group after intratracheal exposure. In fact, the outbreak can be explained by spread of vaccine virus from vaccinated to unvaccinated chickens, the virus perhaps gaining virulence as it passes from bird-to-bird.

As outlined in the *Introduction*, previous study suggested a possible causal role for vaccine viruses in ILT infection, perhaps, as a result of reversion of vaccine viruses to parental-type virulence (Guy *et al* 1990). The replication and spread of vaccine viruses are potentiated by vaccine administration that fails to provide immunity to all the birds in a flock, for example, via drinking water and spray (Fulton *et al* 2000, Robertson & Egerton 1981). They are determined that these isolates are identical to the chicken embryo origin (CEO) vaccine by restriction endonuclease analysis, thus disease outbreak in a previously vaccinated flock may be due to non-uniform flock immunity from a low-titer vaccine, improper vaccine application, or waning flock

immunity. Also there is an indication that the CEO vaccine virus spreads soon after vaccination (Andreasen *et al* 1989). The ability of vaccine strains of ILT virus to produce long-term lateral infection has considerable implications for the control of the disease as well. It is probable that the ILT vaccine viruses, themselves, include long-term latent ILT infection, which is the potential for subsequent natural spread (Bagust 1986). On the other hand, an increased virulence after sequential bird-to-bird passage of vaccine viruses is observed for CEO virus but not tissue culture origin virus (Guy *et al* 1991). It is indicated that CEO ML ILT vaccine viruses are not stable with respect to virulence properties and thus may causally be involved in disease outbreaks. The ML ILT vaccines may be inappropriate for ILT control, because they may induce latently infected carriers and may be responsible for ILT. Thus, the current ML ILT vaccine is neither ideal nor sufficient to support the development of control programs and most practical research towards improved ILT disease control must be done either by correct administration of current ML ILT vaccine or developing an inactivated vaccine.

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