

# The Early History of Infectious Laryngotracheitis

Morris S. Cover

## EARLY IDENTIFICATION OF LARYNGOTRACHEITIS IN THE UNITED STATES

Laryngotracheitis in the United States may have occurred as early as March 1920. In 1926, outbreaks in California chicken flocks were reported in which fowl pox apparently coexisted. Clinical signs were coughing with expulsions of blood and mucus as well as severe dyspnea (3). Necropsy records of specimens submitted for diagnosis to the Department of Veterinary Science, University of Massachusetts, indicate that a disease resembling infectious laryngotracheitis was observed in 1931 (19). Diagnostic laboratories reported a disease with similar signs during the mid 1920s. As with most emerging diseases, several names were used to identify this condition, including infectious bronchitis (2,3,6,24,25), tracheolaryngitis (36), infectious tracheitis (19), and avian diphtheria (23). There are also references in the literature to chicken flu and Canadian flu. The term infectious laryngotracheitis (ILT) was adopted in 1931 by a special committee on poultry diseases of the American Veterinary Medical Association. A 1931 survey of outbreaks in California reported morbidity of almost 100% and mortality ranging from 0 to 49%. Gasping with the expectoration of mucus and blood was a common symptom.

Initial published reports of laryngotracheitis from various countries are depicted in Table 1. The chicken is the primary natural host for infectious laryngotracheitis virus (ILTV). All ages are susceptible but it appears that older susceptible flocks demonstrate more severe clinical disease on initial exposure. Pheasants and a pheasant-bantam cross were shown to be susceptible (29). The disease could not be reproduced in turkeys, ducks, starlings, quail, pigeons, or sparrows (44). Other avian species reported to be resistant were crows, doves, and guinea fowl (5,10). The following animals are refractory, rabbits, guinea pigs, white rats (5,44), and the Swiss mouse (38).

## SIGNS AND LESIONS

Clinical signs of this disease are usually acute in onset with high flock morbidity and variable mortality. Nasal discharge, lacrimation, and moist rales are followed by coughing and gasping. In the later stages of the disease, severe dyspnea occurs, characterized by an obvious extension of the head and neck during inspiration. On expiration, the head falls down, frequently resting on the floor. This sign is caused by obstruction of the trachea and glottis by desquamated epithelium and exudate. Chickens having large amounts of tracheal exudate with dyspnea produce a whistle or stetorous sound and are designated as "callers." In acute cases and depending on the virulence of the virus, expectoration of blood may occur. In some affected flocks, this sign was so prevalent that the walls and equipment were spattered with dried blood. Today, the clinical picture of the disease in many cases does not include the acute signs, which may be a feature of a virus of low pathogenicity or reflect immunity stimulated by vaccination.

On postmortem examination, acutely affected birds show mucoid inflammation followed by necrosis and desquamation of the tracheal mucosa. Yellow caseous exudate is present, which at times forms a hollow cast and may progress to occlude the tracheal lumen resulting in asphyxiation. The microscopic tissue lesions were studied as early as 1931. Chickens were artificially infected by inoculation of the virus intratracheally, intranasally, intraocularly, into the cleft palate, and by contact. In each case, typical tracheitis was produced as well as conjunctivitis and a nasal discharge. The type and severity of the lesions varied greatly. In some cases, there was only a mild tracheitis, in other cases (45) clotted blood was present, and in still others a large amount of exudate was present. Variation in the pathogenicity of the ILT virus isolates has been reported by several workers (12,13,42). Many isolates made in the 1930s through 1960s caused only mild signs and low

Table 1. Infectious laryngotracheitis identifications in the world.

Location	Year	Reference
Canada	1925	23
Australia	1935	43
Britain	1935	16
Sweden	1940	34
Holland	1946	50
Poland	1948	35
Germany	1959	17
Finland	1965	39

morbidity and mortality. It has been postulated that the virus persists in chicken populations in a subclinical form with resurgence when environmental conditions or immunosuppression occur. The cyclic appearance of the disease in some areas including the Delmarva peninsula possibly reflects increases in the proportion of susceptible birds in the population exceeding the outbreak threshold. With superimposition of movement of flocks or other factors favoring transmission of the virus, clinical outbreaks occur.

#### EARLY ATTEMPTS IN ISOLATION AND IDENTIFICATION

Early attempts to isolate an etiologic agent were unsuccessful. In some clinically affected flocks, *Pasteurella* sp. was isolated and in other cases pox virus was identified. These agents did not fulfill Koch's postulates and were rejected as the causal organism. Laryngotracheitis virus was first demonstrated in 1930 (6). These findings were confirmed by Dr. J. R. Beach (4). Dr. C. S. Gibbs completed a number of filtration experiments to demonstrate the viral etiology of laryngotracheitis (19). He found that viruses obtained from acute field cases consistently passed the Berkfield and Seitz filters. He successfully reproduced the disease in pullets, cockerels, and chickens with the filtrate. He reported that some of the viruses were retained by the Berkfield N filter but virus was not present in the filtrate passing through the Berkfield W filters. By using viruses from chronic cases, he found that potency was lost with serial passage. In 1935 it was reported that this virus was between 45 and 85  $\mu$ m (22). It was only in 1963 that the agent was characterized as a herpes-type virus (15).

The lesions produced by the ILT virus in the chorioallantoic membrane of the 10-day-old chick embryo were first described in 1934 (11). The isolated foci produced were described as having an opaque raised edge and a gray central area of necrosis. Later (1937), a report indicated that this lesion may be seen as early as 48 hours following inoculation on the chorioallantoic membrane (8).

#### EVOLUTION OF VACCINATION PROGRAMS

In the early 1930s, research had not progressed to the point where vaccination was available as a means to control ILT. Following isolation of the virus, experiments were initiated to provide the industry with a means of protecting their flocks.

The following very interesting quotation from a report by Dr. C. S. Gibbs (21) documents his experience in vaccinating birds in an acute laryngotracheitis outbreak.

If infectious laryngotracheitis has already appeared in a small portion of the flock and its virulence is satisfactory for immunization, then autogenous vaccine should be used. Autogenous vaccine may be prepared as follows: Take a bird that has just died or one that is very sick and kill it. Lay the dead bird on a table, box, or barrel, on its back, and beginning at the beak slit open the skin of the neck with a pair of scissors, exposing the windpipe to the wish-bone. Carefully dissect the windpipe from the other tissues, taking care to get as much of it as possible. Now slit the windpipe open, beginning at the larynx and cutting clear through to the other end; and, by means of a small knife—a paring knife, a pen knife, or a scalpel—scrape the exudate from the exposed larynx and trachea and put it in a bowl or mortar. After the desired amount of exudate has been secured, grind or triturate it with a smooth stick or pestle, adding a little cold water or a mixture of glycerine and saline until a thin, paste-like mass is formed. This is the vaccine. If it has been properly prepared from birds sick or dead of infectious laryngotracheitis, it should be more virulent than any that can be purchased because it is fresh.

Furthermore, it is autogenous, and should be specific for the particular disease that the birds are affected with. In order to get the best results, fresh vaccine should be made from the sick birds every two hours and any that is left over should be destroyed.

This procedure provided some protection to flocks diagnosed with ILT when the autogenous

vaccine was applied to the cloaca. He provided specific instructions for preparing this material on the farm.

The following is an account of an early endeavor to control the disease by vaccination using only the resources available at the farm. Gibbs indicated that he did not have his vaccination equipment with him as the trip was taken for an entirely different purpose, and it was necessary to improvise (21).

In Gibb's words,

Applicators were split from kindling in the wood pile, cotton for making swabs was secured from the family medicine closet, scissors from the sewing room, a bowl and a little water from the kitchen. With this crude equipment, the writer, the owner and a man hired for the occasion set out to save the chickens on the range from dying of infectious laryngotracheitis. Thirty of the pullets showing marked symptoms of infectious laryngotracheitis were sacrificed to make vaccine, and the 800 chickens on the range were vaccinated in the cloaca and bursa of Fabricius. Five days later the flock was examined for takes.

The most favorable time for reading "takes," represented by a pseudomembraneous inflammation of the cloacal mucosa, was between the 4th and 5th days following vaccination (21). Of the birds vaccinated, 88% showed "takes" without signs of disease, 8% showed "takes" with respiratory signs, 3.5% had neither "takes" nor symptoms, and 0.5% showed signs without "takes." All birds in the flock remained free of the disease throughout the year except for the four individuals that did not show either "takes" or signs following vaccination.

This account provides a perspective of the early activities in the prevention of laryngotracheitis by vaccination. Credit must be given to these early efforts by veterinary scientists to provide the industry with a means to protect their flocks from a new disease with a serious impact on production and livability.

It was originally suggested that it was impractical to prepare vaccine in the laboratory on a large scale with a satisfactory level of antigenicity (20). The standard of antigenicity was assayed by inoculating a batch of susceptible birds by the intratracheal route. If most birds show signs of laryngotracheitis and some of them die within 3 days, the vaccine would be considered effective. It was recommended that if the vaccine proved unsatisfactory, the test

birds should be destroyed and the premises thoroughly cleaned and disinfected before preparing subsequent batches.

There are other reports from avian pathologists in 1933-34 using similar crude autogenous vaccines to reduce mortality in chicken flocks (8,10) infected with ILT.

The following quotation from Gibbs (20) summarizes the evaluation of the vaccination procedure under field conditions.

The success of the vaccination depends upon the number of takes. Four days after vaccination the birds should be examined for takes. In checking up on the number of takes, the same procedure can be followed as in vaccinating the birds. The person reading the takes should have a supply of swabs handy and revaccinate any birds in which takes did not occur with exudate from birds which show good takes. A take has occurred in those birds in which the mucous membrane of the cloaca is moist, inflamed, or covered with pseudomembrane.

Vaccination is a medical treatment and medical treatments have their limitations. If the limitation of infectious laryngotracheitis vaccination are appreciated, cloacal and bursal inoculation may be successfully accomplished on the poultry farm and serious loss from the disease prevented.

The earliest studies were directed at determining if chickens could be successfully immunized against infectious laryngotracheitis. Attempts to vaccinate birds included the introduction of the virus by a route other than the respiratory tract. Oral administration of the unfiltered virus in gelatin capsules did not elicit any clinical signs and did not protect against challenge by a homologous isolate (20). A Seitz-filtered virus administered into the wing vein stimulated some protection (20). Intratracheal challenge indicated that low dilutions of virus produced only partial immunity and but more potent vaccines were fatal. It was suggested that the virus was carried to various organ systems after administration where it could cause clinical disease. In 1932, Dr. C. B. Hudson and Dr. F. R. Beaudette at Cornell University reported on trials using the cloaca as a vaccination site (30,31).

In 1933, Gibbs followed this lead, reporting that the virus did not persist in the cloaca (bursa of Fabricius) although it did produce an inflammation. He also reported that challenge studies showed a waning in immunity. Some of

the vaccinated birds were shown to be chronic carriers of ILT, a characteristic of herpes viruses. Carriers were identified among flocks in acute cases of the disease and in field-vaccinated birds, especially the individuals that reacted strongly to the vaccine (19). It was observed that in naturally affected birds immunity was permanent, whereas artificially acquired immunity was of variable duration positively correlated to the severity of the vaccine reaction. Studies in 1931 confirmed that the virus did not persist in the cloaca for more than a few days and only survived 2 days outside the bird. In freezing weather, viability outside the host was extended to 5 days (19).

#### **ARTIFICIAL PROPAGATION OF THE VIRUS**

By 1934, workers at Kansas State College indicated that the ILTV could be easily propagated on the chorioallantoic membrane of embryonated chicken eggs (8,10). It was suggested by Dr. C. A. Brandly that large quantities of pure virus could be produced economically to vaccinate chickens (9). Studies conducted in 1936 by Dr. F. M. Burnet furthered the knowledge concerning plaque formation on the chorioallantoic membrane by the ILTV. He also reported that the virus was not immediately inactivated by specific antiserum and that the number of plaques produced was proportional to the concentration of the virus (12).

In 1964, a tissue culture-modified ILTV was developed to be used as a possible vaccine (18). Following vent application immunity was present in 3–4 days when assessed using intranasal challenge. When applied by the intraocular route, immunity was established in 4–5 days. Birds vaccinated intraocularly showed protection for 20–22 weeks.

#### **EFFECTIVENESS OF VACCINATION PROCEDURES**

In 1960, it was reported that the immune status of the parents did not influence the response of chicks to vaccination (14). This investigation reported that birds 2 weeks of age or younger did not fully respond to a vaccination as determined by challenge.

The need to abrade the cloacal membrane when vaccinating chickens was investigated in

1958 (26). This research indicated that the vent drop method elicited an immune response and abrasion of the mucosa was not essential to obtain satisfactory immunity. The suggested advantages of the drop procedure include uniform dosage bird to bird; uniformity of application; elimination of fecal contamination; ease, rapidity, and cleanliness of the procedure; elimination of injury to the bursal mucosa; and ease and speed of application in young birds. However, it was reported that the immunity resulting from vaccinating chicks at 3, 10, and 21 days of age was not durable.

In 1959, it was reported that Newcastle disease, infectious bronchitis, and infectious laryngotracheitis vaccinations could also be effectively administered by the vent drop route (51). Satisfactory immunity was established against these three diseases when the viruses were administered singly or in combination with each other.

Field experience with breeders and layers indicated that a long-lasting immunity was not stimulated by vaccination against infectious laryngotracheitis. After testing several revaccination procedures in 1960, the eye drop method was shown to produce the best response. Other techniques such as vent drop, vent brush, and intranasal drop did not give acceptable results (27). Infraorbital sinus challenge confirmed that birds vaccinated by the vent brush or drop method at 4 and 8 weeks of age showed a high degree of susceptibility when challenged at 16 weeks of age by the infraorbital sinus route. These results indicated the fallacy of making claims for life-long immunity following vaccination. Revaccination by the eye drop procedure showed promise for stimulating increased resistance, as shown by serologic response by the serum neutralization test and protection from infraorbital challenge using 0.1 ml of reconstituted vaccine of a homologous strain (27). An avirulent broadly antigenic ILT vaccine was administered to chickens over 10 weeks of age by the eye drop route. It was shown to be adequately immunogenic (47). Cockerels vaccinated by the conjunctival route were refractory for as long as 372 days to an intrasinus challenge of a homologous virus. One-day-old chickens immunized by the conjunctival route with an egg-propagated commercial vaccine showed a transient ocular discharge in a small percentage of the birds. Chal-

lenge was accomplished by intratracheal instillation at 26, 28, and 35 days of age with an egg-propagated commercial vaccine, which indicated acceptable protection (48). Immune response following intraocular vaccination under field conditions protected birds from intrasinus challenge and resulted in protective levels of neutralizing antibodies for 49 weeks (46).

An egg-propagated mild ILTV used as an intraocular vaccine protected subjects 6 days after administration with a duration to intrasinus challenge as long as 20 weeks. This vaccine spread to susceptible contacts (1).

A comparison was made between artificially modified virus and a strain of low virulence. Both the modified virus and the apathogenic strain were avirulent by the intraocular or intratracheal route (40). Both viruses spread to susceptible contact birds, which resisted challenge.

The drinking water route of vaccination against ILT was investigated in 1969 in Israel (40,41). In a laboratory trial, 3- to 6-week-old chicks received a vaccine modified by sequential passage in embryonated chicken, duck, and turkey eggs. Intratracheal challenge 2, 4, and 6 weeks after immunization demonstrated protection. The modified virus spread to unvaccinated contact chicks, producing protective antibodies in half of the group. In parallel field trials severe reaction to this drinking water-type of vaccination was recorded, with mortality exceeding 5%.

Vaccination by the feather follicle route was attempted by several workers in 1947. Seven of eight commercial vaccines produced satisfactory immunity when applied to the feather follicle. However, the wing-web stick procedure was of no value. These workers did not compare the immunity produced with that of the cloacal method (37). It was noted that this technique required more vaccine than the cloacal method. In 1959, Dr. S. Hunt adapted a strain of ILTV for feather follicle vaccination (32).

### TRANSMISSION

Direct transmission (carrier to susceptible bird) of the infectious laryngotracheitis virus has been accepted since the report by Dr. C. S. Gibbs (19). Early work on the transmission of ILT showed that it was not possible to reproduce the disease by administering capsules con-

taining the virus *per os*. Dried material containing the virus sprinkled over litter caused the disease in susceptible chickens, which showed typical signs (20). Beaudette suggested it was unlikely that insect vectors played any part in transmission (7). The U.S. Livestock Sanitary Association stated that the virus may be carried on the hands and clothing of humans and on poultry equipment of any kind (49). Indirect transmission by crates, equipment, and by free-living birds was implicated in the late 1950s (28). Transmission to five farms in Connecticut was attributed to contaminated clothing that was not appropriately disinfected. Other sources of infection included a farm dog, crows, and rats. Indirect transmission occurs when the respiratory secretions are fresh and directly disseminated from sick to susceptible birds (33).

In 1984, Canadian workers studied the methods of spread of infectious laryngotracheitis virus in chicken flocks. The results of this work suggested that farms with previous infections were more likely to have future outbreaks. Sanitation procedures of people were related to outbreaks and the air inlet location was a feature. For example, barn inlets facing north had higher risk ratios. This report suggested that the virus may be wind-borne (52).

### GENERAL COMMENTS

It became obvious as literature was reviewed and interviews and discussions proceeded that Dr. C. S. Gibbs was prominent as an early investigator and reporter in ILT. His initiative in developing vaccination procedures to control infection in flocks was responsible for protection of the industry, and for furthering an understanding of infectious laryngotracheitis. Others who were also notable in the early investigations included Beach, Beaudette, and Brandly. These first attempts to provide protection against the disease, although crude and imperfect, show the ingenuity and inventiveness of scientists such as Dr. Gibbs.

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