

MASSACHUSETTS
AGRICULTURAL EXPERIMENT STATION

December, 1931

Infectious
Laryngotracheitis
Carriers

Dhananjayrao Gadgil Library



GIPE-PUNE-047534

The investigations of the past year at this Station have centered largely around carrier problems and control measures. While the goal has not yet been reached, important advances have been made, and the disease can now be handled under laboratory conditions more effectively than it was a year ago. The results contained in this paper are in no sense complete, but are published in the hope that poultrymen and others interested in the control of infectious laryngotracheitis may find in them some practical applications under field conditions.

MASSACHUSETTS STATE COLLEGE

AMHERST, MASS.

7351:42.7885

47534 G1

INFECTIOUS LARYNGOTRACHEITIS CARRIE

By Charles S. Gibbs,

Research Professor of Veterinary Science

Chronic carriers of the virus of infectious laryngotracheitis¹ at the beginning of this study, because cleaning and disinfecting moving apparently recovered birds to new quarters had failed to prevent disease in more instances than could be accounted for by accident. Moreover, on several poultry farms, the annual appearance of the disease in pullets and cockerels brought in from the range in the fall and winter strongly indicated the presence of carriers among the adult birds. In an examination of 144 birds by means of intratracheal swabs, two birds were observed (Gibbs, 1931) eliminating virus longer than usual without showing any symptoms of disease. Although these observations did not reveal the existence of chronic carriers, they signified the possibility of such carriers. The investigation was continued until the 14 chronic carriers described in this bulletin were found and studied.

Since these experiments on carriers of infectious laryngotracheitis were completed, Komarov and Beaudette (1931) report the finding of chronic carriers by the same method. While these investigators did not state the total number of birds examined, they concluded: "Finally, in a population of 25 birds which had infectious bronchitis 16 months previously, 3 carriers of the virus were determined."

For some time chronic carriers of infectious laryngotracheitis have been suspected. Now experimental evidence from two independent sources shows that such carriers actually exist.

Experiments on subjects closely related to chronic carriers, such as mechanical carriers, the channel of infection, the viability of the virus under natural conditions, and practical suggestions for the control of the disease are included in this bulletin.

Mechanical Carriers

Experiments have been conducted with sparrows and mice as possible carriers of infectious laryngotracheitis. Thus far the results indicate that these pests are not important in the transmission of the virus, as neither of them is susceptible to the disease. However, both sparrows and mice served as mechanical carriers when the feathers and hairs were smeared with fresh exudate from the tracheas of birds dying of acute infectious laryngotracheitis, a condition not likely to occur under natural circumstances. In another experiment two cages, one containing diseased birds, the other healthy birds, were placed twenty inches apart and connected with a wire runway, arranged so that only the sparrows and mice could pass from cage to cage, as shown in Figure 1. During the day the sparrows were seen passing back and forth in search of feed. At night the mice got

¹ This name was suggested by Dr. H. J. Stafseth, at the 1929 Conference of State and Federal Research Workers in Animal Diseases; and it has been recommended by the Special Committee on Poultry Diseases appointed to report at the 1931 meeting of the American Veterinary Medical Association. In order to assist the movement for standardized nomenclature in poultry diseases, the name infectious laryngotracheitis is, therefore, used in this bulletin for infectious tracheitis and infectious bronchitis.

into the feed in both cages, clawing it over and picking out pieces to nibble. Since the susceptible chickens showed no signs of the disease, the mice and sparrows did not serve as mechanical carriers under these conditions.

Ten pigeons from two widely separated flocks were inoculated intratracheally with a virulent strain of the California infectious laryngotracheitis virus. The pigeons were not affected by the disease, although two pullets inoculated with the same virus at the same time died in five days. The virus lived only a few hours in the tracheas of the pigeons, for after one day tracheal exudates from them did not infect susceptible chickens. Beach (1931) was unable to reproduce the disease by inoculation in pigeons.

A goose sent to the diagnostic service, apparently suffering from infectious laryngotracheitis, was killed and necropsied. The gross and histopathological lesions indicated infectious laryngotracheitis, but four chickens inoculated intratracheally with fresh tracheal exudate failed to develop the disease, although they readily succumbed to a natural infection later. Graham and Thorp (1931) have reported a similar experience with a wild goose. As yet it is uncertain whether or not geese are susceptible to the disease or act as carriers of the virus.

An attempt was made to determine whether man could serve as a mechanical carrier of infectious laryngotracheitis. In this experiment 24 susceptible pullets and cockerels were placed in separate cages. The cages were set on three double-deck stands well isolated from one another. The birds on the first stand were numbered A1, A2, A3, A4, A5, A6, A7, and A8, respectively; those on the second stand, B1, B2, etc.; and those on the third stand, C1, C2, etc. A1 and B1 were inoculated intratracheally with a virulent strain of infectious laryngotracheitis, while C1 was kept as an uninoculated control. Six days after inoculation both A1 and B1 showed symptoms of infectious laryngotracheitis and were emitting tracheal exudate tinged with blood during paroxysms of coughing. Apparently the conditions were right for the next step in the experiment. Accordingly, the experimenter carefully washed his hands and arms in warm, 2 per cent compound cresol solution, finally rinsing in running tap water, and drying with paper towels. He then put on a recently laundered laboratory coat and handled the pullets and cockerels in the A cages as in a poultry judging contest, beginning with A1 and taking each bird consecutively. After disinfecting hands and arms and changing to another laboratory coat, he handled the birds in the B cages just as he had those in the A cages. The same procedure was repeated for the birds in the last group, beginning with C1. All of the pullets and cockerels in the A cages, five in the B cages, and none in the C cages came down with infectious laryngotracheitis within the period of incubation for this disease, indicating that man may serve as a mechanical carrier of the virus when the excretions are fresh and carried directly from sick to healthy susceptible birds. Later the controls (C birds) and B5, B7, and B8, which did not take the disease by handling, were inoculated intratracheally with virulent tracheal exudate from A2 and A3. B8 and all of the controls came down with infectious laryngotracheitis in due season and died. B5 and B7 did not contract the disease, and were later found to be resistant to it.

Acute Cases

The most important distributor in infectious laryngotracheitis, as in most acute infectious diseases, is the recently infected individual. The virus is nearly always found in the tracheal exudate during the acute stages, and it has been demonstrated in the internal organs in a limited number of cases in which an especially virulent virus was used and special laboratory methods employed.

The intravenous injections of filtered tracheal exudates into susceptible birds indicate that the living virus can circulate in the blood in sufficient quantities to cause general infections, but immune bodies are developed in this tissue, so that it has been impossible to demonstrate its presence by direct methods.

The nervous system is apparently rarely affected, although the hen shown in Figure 2 contracted the disease naturally and developed chorea from which it never fully recovered. The hen was kept under observation for six months. At necropsy a hemorrhage was found on the ventral surface of the cerebellum. A small blood vessel had ruptured in the cerebellar tissues and blood had flowed into the space between the meninges and the cerebellum, forming a clot. This rupture and blood clot appeared like those occasionally found in the respiratory, circulatory, and visceral organs of fowls dying of acute infectious laryngotracheitis. A slight infiltration in the brain tissues around the clot indicated that the blood was being slowly absorbed. In another set of experiments two chickens were successfully inoculated with infectious laryngotracheitis from the brain of a pullet dying of the disease. Blood clots were not found in this pullet, although the brain material was preserved by Swift's (1921) method and other chickens were inoculated later, confirming the infectiousness of the tissues examined.

Birds showing visible symptoms of infectious laryngotracheitis are called acute cases. (Figure 3.) An attempt has been made to determine the length of time acute cases eliminate virus, because of the importance of such knowledge in developing control measures. Since the experiments reported in this bulletin were started in November, 1930, individual tests have been made on 504 birds, with ten viruses from widely separated places in Massachusetts, four from California, and four from New Jersey. The number of cases of acute infectious laryngotracheitis among the birds studied, and the number of days during which they eliminated virus were as follows:

Virus Used	Resistant Birds <i>Number</i>	Acute Cases <i>Number</i>	Period of Eliminating Virus		
			Shortest <i>Days</i>	Longest <i>Days</i>	Average <i>Days</i>
Massachusetts strains	11	269	4	18	6.73
California strains	4	108	3	11	6.14
New Jersey strains	2	110	2.8	8	5.25
Totals and average	17	487			6.27

Chronic Carriers

Birds which show no visible symptoms of the disease and yet eliminate virus are called chronic carriers. The chronic carrier stage was not found at all ages, for 160 chickens, inoculated when two weeks old and younger, were included in these studies and when tested later no chronic carriers were found among them. Chronic carriers were found among the pullets, cockerels and older birds, as follows:

Virus	Chronic Carriers
Massachusetts strains	9
California strains	3
New Jersey strains	2
Total	14

The number of days the 14 chronic carriers eliminated sufficient virus at weekly tests to inoculate susceptible chickens, up to the date this paper was written, were as follows:

Carriers Number	Virus	Days
V 2270	Massachusetts	378*
V 2290	California	234
V 2320	Massachusetts	225
V 2321	New Jersey	211
V 2370	Massachusetts	252
V 2383	Massachusetts	200
V 8225	California	196
V 8277	New Jersey	154
V 1200	Massachusetts	182
V 1231	Massachusetts	140
V 1260	Massachusetts	165
V 1269	Massachusetts	98
J 3835	California	120
J 3393	Massachusetts	67

* V 2270 was still a chronic carrier at the time this paper was written.

The results of the above experiments indicate that a small number of all birds in a flock surviving infectious laryngotracheitis are liable to become chronic carriers for an indefinite period. The longest period was not determined, but the shortest was 67 days. The less virulent Massachusetts virus produced the greatest number of carriers and the most virulent New Jersey virus the least. It is evident that the more virulent strains produced the smaller number of chronic carriers. The reason for this is unknown, although it may be readily supposed that these strains killed some of the birds that would have become chronic carriers if they had been infected with a less virulent virus. When a bird once became a carrier it invariably remained so for two months or longer up to an indefinite period. Chronic carriers for shorter periods were not found in these experiments, although birds harboring the virus may have existed and not been detected by the laboratory methods used.

Two Types of Chronic Carriers

Two types of chronic carriers, characterized in this bulletin by the terms "regular" and "irregular," were found among the birds examined. The regular carriers eliminated the virus at weekly tests as long as they remained carriers, while the irregular carriers failed to eliminate virus at two or more tests during the carrier stage. Possibly the birds should have been classified as constant and intermittent carriers, but on account of the cost involved in providing chickens for making the tests it was impossible to study the constancy and the intermittency of the elimination of the virus more closely. The number of regular and irregular carriers as detected by weekly inoculations of tracheal exudates from the chronic carriers into the tracheas of susceptible chickens were as follows:

Virus	Carriers	
	Regular	Irregular
Massachusetts strains	7	2
California strains	2	1
New Jersey strains	2	0



Figure 1. Wire Cages Connected by Runway—used for the mouse and sparrow experiments.



Figure 2. Choreia in a Fowl, following an attack of Infectious Laryngotracheitis.

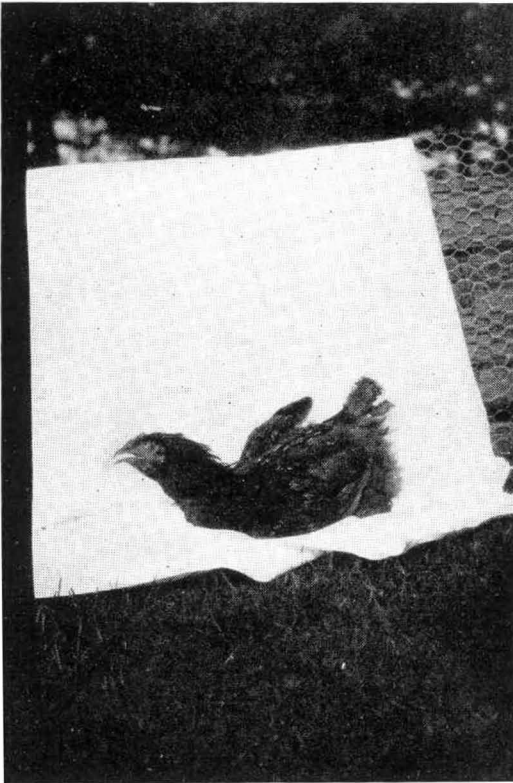


Figure 3. An Acute Case of Infectious Laryngotracheitis.

As soon as the chronic carriers ceased to eliminate virus from the trachea, they were killed, and the internal organs were tested for the presence of virus by direct and indirect methods. The virus was not found in any of the internal organs examined; but it should be borne in mind that the total number of carriers was limited to 14, and that it is difficult to demonstrate the virus of infectious laryngotracheitis in the tissues of birds by laboratory methods, unless it is present in large quantities.

Experiments with Dried Infectious Laryngotracheitis Tissues and Exudates

The cages used in the experiments with man as a mechanical carrier were disinfected by submerging for 12 hours in a tank containing 3 per cent cresol solution, numbered, and returned to the stands. Twenty-four pullets and cockerels were placed in the cages. For a month the birds in the A cages were fed desiccated diseased tracheal tissues and exudates, but none of them developed the disease. At the end of the month the birds were inoculated, intratracheally, with tracheal exudate from a pullet dying of the disease and all were found to be susceptible.

The litter in the B cages was thoroughly dusted with some of the same material that was fed to the birds in the A cages, except that it was ground to a fine powder. All of these pullets and cockerels developed the disease in three to seven days after the first dusting, and six of them died.

The birds in the C cages were fed powdered infectious laryngotracheitis material in gelatine capsules, but did not take the disease. Later their tracheas were swabbed with powdered tissues and exudates and all of them developed the disease and died.

These laboratory investigations indicate that the virus is not likely to cause disease in susceptible pullets and cockerels unless it is in some form that will readily attach itself to the mucous membrane of the respiratory tract. In other words, the channel of infection in laryngotracheitis seems to be the respiratory tract, and it makes little difference whether the virus is moist or dry; for duplicate feeding experiments with moist tracheal tissues failed to infect susceptible chickens while the spraying of liquid exudates invariably produced the disease.

The Viability of Infectious Laryngotracheitis Virus Under Natural Conditions in a Poultry House

The preceding investigation indicated that the environment in some poultry houses might be conducive to the preservation and spread of infectious laryngotracheitis, especially in cold weather. An experiment was therefore planned to determine the viability of the virus under these conditions. In this experiment small pieces of epithelium, pseudomembrane, and tracheal exudate were scraped from the tracheas of fowls dying of the disease, and placed in serological test tubes which were plugged with cotton. Then the tubes were buried in the litter on the floor of the poultry house. Every day during the course of the experiment, except Saturday and Sunday, a tube was dug up and the contents examined and used to inoculate chickens susceptible to infectious laryngotracheitis.

The results of this experiment indicate that the virus was not preserved for any great length of time under the conditions in the litter of the poultry house, except during the latter part of January and the first part of February

when the temperature and relative humidity were comparatively low. The conditions then prevailing were more favorable, and the virus was preserved in a viable condition in one trial for six days and in another for nine days. All of the virulent pieces of tissues and exudates had dried out considerably, but not enough to preserve them indefinitely.

The location of the tubes in the litter and in the poultry house was important; for the virus in the tubes placed in moist and sunny places, such as near the drinking fountain and in front of the windows, lost its virulence in three days. The virus in tubes buried in the corners, under the roosts, and under the laying boxes remained virulent for longer periods.

It was impossible to measure the contribution of other factors that may have been involved in the partial desiccation and prolongation of the virulence of the tissues and exudates in the test tubes. Possibly the dry, dusty litter absorbed the moisture, and such aerobic bacteria and moulds as grow at low temperatures removed some of the oxygen, thus making the atmospheric conditions favorable for a partial preservation of the viruses used in these experiments.

Practical Applications

When infectious laryngotracheitis has existed in a flock and the recovered birds are kept for breeding purposes, there is reason to suspect that some of them may be carriers, and it is very unwise to mix any of the pullets and cockerels coming in from the range in the fall with the breeders. The two flocks should be kept entirely separate. The old hens and roosters should be disposed of as soon as the breeding season is over, and the premises which they have occupied thoroughly cleaned and disinfected. In this way the cycle of infection as it occurs on many poultry farms should be broken and the disease should disappear from the premises.

Terminal disinfection is important in poultry management and should receive more attention than is ordinarily given to it. Terminal disinfection has to do with cleaning up after an outbreak, or after disposing of birds among which carriers are suspected. Before disinfection can be intelligently applied something should be known about the virus, bacteria, or parasite it is desired to kill. It is already known that infectious laryngotracheitis is caused by a filtrable virus (Beach, 1930; Beaudette, 1930; Gibbs, 1931; Graham, Thorp and James, 1931). Experiments show that this virus is easy to destroy outside of the bird. Ordinarily it does not live longer than 24 hours at 68° F. (20° C.) when exposed to light and air, or 3 or 4 days when placed in an ice box. If moisture and air are removed by means of a vacuum pump, and the dehydrated virus is kept in a sealed desiccator just above the freezing point, its virulence is often maintained for several months; but these conditions are unusual, and seldom, if ever, found in a poultry house.

The virulence of the virus in tracheal exudates and possibly in other secretions from diseased birds may be prolonged in the litter of poultry houses in winter. While factors responsible for this prolongation are not well understood, it is quite likely that constant low temperature, low relative humidity, dry environment, and overcrowding are largely responsible for the spread of the disease in poultry houses.

With these facts in mind it is possible to understand why it is very difficult to control infectious laryngotracheitis in winter. At this time of year the virus is likely to be more resistant than it is at other seasons. The disinfectant is apt

to freeze and fail to reach all of the places intended. In addition, colds and roup are likely to appear as complicating factors due to increased draughts and dampness. Usually the best time to clean up after an outbreak of infectious laryngotracheitis is in the summer. All litter should first be removed from the house and burned. Then the house should be thoroughly washed with hot water and disinfected with one of the "Permitted Disinfectants."² The liquid should be sprayed with a force pump, or rubbed into all cracks with a good stiff broom. After standing for a week with doors and windows open to admit light and air, the premises should be free of infectious laryngotracheitis.

Summary

1. Sparrows and mice are not important mechanical carriers of infectious laryngotracheitis.
2. Ten pigeons inoculated intratracheally with virulent strains of infectious laryngotracheitis failed to contract the disease, or to carry the virus in their tracheas for any great length of time.
3. Man may serve as a mechanical carrier of the virus if he handles birds in the acute stages of the disease and directly picks up susceptible chickens, pullets, or cockerels.
4. Acute cases eliminate virus as long as they live, up to 10 or 15 days, or until they show no visible symptoms of the disease.
5. Fourteen chronic carriers out of 504 birds tested eliminated virus for indefinite periods.
6. The number of chronic carriers in a flock surviving an attack of infectious laryngotracheitis depends upon the size of the flock and the virulence of the virus.
7. If the birds used for breeding purposes have had infectious laryngotracheitis and the pullets and cockerels have remained free of it, the two flocks should be kept separate in order to break the cycle of infection and get rid of the disease.
8. The respiratory tract seems to be the most important channel of infection.
9. Usually the best time to clean up and disinfect after an outbreak of infectious laryngotracheitis is in the summer.
10. It should be borne in mind that the control measures suggested in this bulletin are based upon a laboratory study of infectious laryngotracheitis, and it is too much to expect that they will meet all situations arising in poultry practice without some adaptation or modification.

² A list of the permitted disinfectants may be procured by application to the United States Department of Agriculture, Washington, D. C.

References

- Beach, J. R. 1930. The virus of laryngotracheitis of fowls. *Science* 72:633-634.
- Beach, J. R. 1931. Personal communication to W. R. Hinshaw, in A survey of infectious laryngotracheitis of fowls. *Calif. Agr. Expt. Sta. Bul.* 520, p. 26.
- Beaudette, F. R. 1930. Bronchitis in poultry. *N. J. Agr.* 12 (5):3-4.
- Gibbs, Charles S. 1931. Infectious trachitis. *Mass. Agr. Expt. Sta. Bul.* 273.
- Graham, Robert, Frank Thorp, Jr., and W. A. James. 1931. A filterable virus-like agent in avian laryngotracheitis. *Jour. Amer. Vet. Med. Assoc.* 78 (n.s.31):506-517.
- Graham, Robert, and Frank Thorp, Jr. 1931. A laryngotracheitis syndrome in a wild goose associated with pneumomycosis. *Jour. Amer. Vet. Med. Assoc.* 79 (n.s.32):90-94.
- Komarov, A., and F. R. Beaudette. 1931. Some observations on the distribution of virus in the body of birds affected with infectious bronchitis with special reference to the carrier state. *Poultry Sci.* 10 (7):391.
- Swift, Homer F. 1921. Preservation of stock cultures of bacteria by freezing and drying. *Jour. Expt. Med.* 33:69-75.

Acknowledgments

In conclusion, the writer wishes to extend his sincere thanks to Dr. J. R. Beach of the University of California and to the Rockefeller Institute for Medical Research for contributing the California and New Jersey viruses of infectious laryngotracheitis used in this study.