MASSACHUSETTS
AGRICULTURAL EXPERIMENT STATION

Bulletin No. 311 July, 1934

Infectious Laryngotracheitis Vaccination

By Charles S. Gibbs

The loss from infectious laryngotracheitis in poultry flocks in the State of Massachusetts is a matter of much economic concern. An interest in the control of this disease has resulted in field and laboratory experiments to develop a satisfactory method of producing immunity through vaccination. The methods and conditions under which such vaccination is practicable are here presented.

MASSACHUSETTS STATE COLLEGE
AMHERST, MASS.

Figure 1. The Proper Way to Hold a Bird for Vaccination.

Figure 2. The Bursal Groove and the Aperture Leading into the Bursa of Fabricius, Exposed for Vaccination.

INFECTIOUS LARYNGOTRACHEITIS VACCINATION

By Charles S. Gibbs,[1]
Research Professor of Veterinary Science

This bulletin is a continuation of a previous study (Gibbs, 1933b) which should be consulted for a knowledge of the immunology of infectious laryngotracheitis as demonstrated up to the present in laboratory and field experiments. Its object is primarily to record the results of certain field experiments which were conducted in Worcester and Norfolk Counties during the summer and fall of 1933 and the winter and spring of 1934. Before attempting the field work it was necessary to make further laboratory studies in order to determine, (1) what is the most satisfactory time after vaccination to read takes; (2) what percentage of takes will insure a satisfactory degree of immunity for a vaccinated flock as a whole; (3) whether carriers are produced by vaccination. Other laboratory experiments were necessary after the field vaccinations had been completed, in the search for cloacal and bursal carriers and the study of certain other diseases resembling infectious laryngotracheitis clinically.

LABORATORY EXPERIMENTS

The Most Favorable Time For Reading Takes

It is known that the virus of infectious laryngotracheitis has special affinity for the mucous membrane of the eye, nostril, larynx, trachea, cloaca, and bursa of Fabricius. In these experiments a "take" is indicated by inflammation resulting from the inoculation of the virus into the mucous membrane of the cloaca and bursa of Fabricius.

In order to determine the most favorable time for the reading of takes, 365 birds of various ages were vaccinated and daily observations made. It was found, regardless of age, that takes were fairly evident from the third to the eighth day, and that the fourth and fifth days were the best for reading takes in a vaccinated flock, as shown in Table 1.

A Comparative Classification of Takes

The takes recorded in Table 1 were classified into five groups as shown in Table 2. From the beginning it was realized that any attempt to classify takes in vaccinated birds would have to be on a relative basis because they would grade into each other in such a manner that in many cases no sharp limits of demarcation would be evident. But this experiment had some real value in training the eye and the judgment and in developing precision and speed in reading takes under controlled conditions in the laboratory. Without this preliminary training, the field experiments might not have been satisfactory.

In this study the expression "no visible takes" indicates that the virus had no

[1]The writer extends his sincere thanks to Mr. John H. Vondell of the Poultry Department of the Massachusetts State College, for taking the photographs showing the method of holding birds for vaccination against infectious laryngotracheitis.

visible effect upon the mucous membrane of either the cloaca or the bursa of Fabricius. In these cases, the mucous membrane was inflamed from rubbing, but the inflammation was transient and the mucous membrane was dry, indicating that the condition was due to the mechanical destruction of the epithelium which kept the surface moist. To the pathologist this is known as desquamative inflammation, and it was so called in this experiment.

A poor take was indicated in those cases in which the mucous membrane of the cloaca or bursa of Fabricius, or both, was moist from the exudation of mucous and serous fluids. Sometimes the feathers around the vent were moist and soiled. This was called serous inflammation.

A fair take resulted when the exudate was fibrinous and the mucous membrane showed petechial hemorrhages in the bursal groove and the opening into the bursa of Fabricius. This was designated as fibrinous inflammation.

A good take showed all of the characteristics of a fair take plus a diphtheritic plug in the opening into the bursa of Fabricius and ecchymosis of the mucous membrane of the bursal groove. This is mild diphtheritic inflammation, and a good take differed from an excellent take only in the severity of the inflammation.

An excellent take showed not only a diphtheritic plug in the opening into the bursa of Fabricius, but pseudomembrane on the mucous membrane of the bursal groove similar to that occurring in the larynx and trachea of birds suffering from natural infections. Sometimes hyperemia and hemorrhage of the mucous membrane of the bursal groove and bursa of Fabricius were evident. This condition would be designated by pathologists either as severe diphtheritic or as hemorrhagic inflammation depending upon the condition most evident.

It should be noted that most of the excellent takes occurred in the pullets and cockerels 4–7 months old and that the chickens 1–3 months old were second, while the hens and roosters 8–12 months old were third. This indicates that, under the conditions of the experiment, the best time to vaccinate is between 4 and 7 months of age.

TABLE 1. THE NUMBER OF DAYS AFTER VACCINATION IN WHICH TAKES WERE EVIDENT.

Days after Vaccination	Number of Takes Shown by Birds of Different Ages			
	1–3 months	4–7 months	8–12 months	Total
1	0	0	0	0
2	0	0	0	0
3	10	8	9	27
4	50	57	45	152
5	47	50	46	143
6	10	7	15	32
7	0	0	0	0
8	0	0	0	0
Total Takes	117	122	115	354
No Visible Takes	8	3	10	21
Total Birds	125	125	125	375

TABLE 2. A COMPARATIVE CLASSIFICATION OF TAKES

Number of Birds	Age in Months	No. Visible Takes	Number of Takes			
			Poor	Fair	Good	Excellent
125	1- 3	8	8	15	40	54
125	4- 7	3	1	11	30	80
125	8-12	10	10	25	40	40
375		21	19	51	110	174

Invisible Takes

It should be noted in Tables 1 and 2 that there were 21 birds in which no visible takes appeared. Since the bursa of Fabricius had completely involuted in the hens and roosters and was represented only by a bursal fold, the 10 birds not reacting to the vaccination in this group may have been naturally resistant to the disease. Just what part natural resistance plays in infectious laryngotracheitis has not been fully determined, although there is some evidence to indicate that it may be an important factor in baby chicks receiving nourishment from the yolk-sac and adult birds eight months or older.

In this experiment 200 chickens corresponding to the 1–3 and the 4–7 months old groups recorded in Tables 1 and 2 were vaccinated. The chickens were killed on the fifth day following vaccination, and necropsied for evidence of takes, special attention being paid to the bursa of Fabricius. The results are given in Table 3.

This table shows that six takes invisible to the eye occurred in the bursa of Fabricius in the first group, and three in the second. These numbers correspond approximately to the numbers eight and three indicating no visible takes in Tables 1 and 2.

What Is a Satisfactory Degree of Immunity for a Flock as a Whole?

From the studies reported in Tables 1 and 3, the percentages of takes that would insure a satisfactory degree of immunity for a flock as a whole were computed from the visible lesions as follows:

Number of Birds	Age in Months	Satisfactory Percentage of Takes
225	1– 3	93.8
225	4– 7	97.3
125	8–12	90.0

It was tentatively concluded from this computation that flocks ranging in age from one to three months could be considered satisfactorily vaccinated against infectious laryngotracheitis if 94 percent of takes were secured for the flock as a whole, 97 percent for those four to seven months old, and 90 percent for hens and roosters.

While these conclusions were logical and backed by scientific results, there were other factors such as the duration of immunity and the production of laryngotracheal carriers in vaccinated flocks that might operate under field conditions and have some influence on the final results. The reason for believing that this experiment should not be considered as final is based on a previous study (Gibbs, 1933b) in which it was found that immunity was not lasting in birds receiving small doses of filtered virus intravenously, and that laryngotracheal carriers were produced in flocks by vaccination Just what result would ensue from two such diametrically opposed forces as these operating in a vaccinated flock could only be imagined, since no experimental work had ever been done with such a flock.

With the object in view of determining just what would happen when the immunity began to wear off in some of the birds in a vaccinated flock in which there were carriers, the 70 birds showing poor and fair takes in Table 2 were placed in a colony house with five known infectious laryngotracheitis carriers.

After two months the immunity began to wear off in the vaccinated birds. As soon as any of the birds were observed coughing, sneezing or gasping, the larynx and trachea were swabbed, and the exudate inoculated intratracheally into susceptible chickens. In this way, 17 of the 25 birds observed coughing and sneezing were found to be carriers for indefinite periods of time as shown in Table 4. It was impossible to test the birds not showing acute symptoms of

TABLE 4. LARYNGOTRACHEAL CARRIERS FOLLOWING A REINFECTION OF INFECTIOUS LARYNGOTRACHEITIS IN A VACCINATED FLOCK.

Days After Vaccination	Carriers	Non-Carriers
1	8	17
4	10	15
8	12	13
12	15	10
16	13	12
20	9	16
24	6	19
28	2	23
32	1	24
36	0	25

reinfection at this time because the forces of infection and resistance which were being studied moved so rapidly.

However, further investigation after the relapse had cleared up revealed two chronic carriers among the birds which had not shown any symptoms of infectious laryngotracheitis, except the results of the vaccination. These two birds remained carriers for a period of two months after all coughing and sneezing had ceased in the rest of the flock, and at necropsy showed lesions of infectious laryngotracheitis in the respiratory tract. One had a diphtheritic ulcer in the mucous membrane of the trachea and the other had a similar lesion in the antero-ventral rima glottis. The remaining birds showed no lesions of disease. During this experiment, the birds did not go off feed, or appear seriously affected, although considerable coughing and sneezing occurred during the period of reinfection.

This experiment indicates that the percentage of takes computed from Tables 1 and 3 insured a satisfactory degree of immunity for the flock as a whole, although a mild outbreak occurred later in those birds showing poor takes. However, the susceptible birds contracted the disease from the carriers in a mild form, thus completing their immunity.

Cloacal and Bursal Carriers in Laboratory Experiments

In a previous experiment (Gibbs, 1933b) neither cloacal nor bursal carriers were found in 52 birds five months after vaccination. Such carriers would be important if they exist, for the virus would be located in a region from which it could be most readily transmitted to other birds in the same flock. However, since the cloaca and bursa of Fabricius are subject to comparatively rapid growth and development, it may be that it is impossible for the virus of infectious laryngotracheitis to maintain a foothold in the mucous membrane of these organs to constitute chronic carriers.

TABLE 5. LENGTH OF TIME IN WHICH VIRUS WAS RECOVERED FROM VISIBLE TAKES.

Days after Vaccination	Number of Visible Takes	Number of Birds from Which Virus Was Recovered from—	
		Cloaca and Bursa	Larynx and Trachea
1	0	30	0
2	0	30	0
3	25	30	2
4	30	30	2
5	30	30	2
6	22	25	2
7	15	23	2
8	8	15	1
9	5	8	1
10	3	2	1
11	0	1	1
12	0	0	1
13	0	0	1
14	0	0	1

Since our knowledge on this point is so meager, it was decided to set up one more experiment before attempting field work. In this investigation 30 pullets and cockerels three months of age were vaccinated in the usual manner and most of the takes appeared on the fourth and fifth days as shown in Table 5. The virus was present in the cloaca and bursa of Fabricius of all the birds the first four days, after which it began to disappear in some of the birds. At the end of the eleventh day, the virus had entirely disappeared from the cloaca and bursa of Fabricius of all of the birds. Two of the birds, however, developed natural attacks of the disease, the virus appearing in the respiratory tract on the third day and remaining in one case for five days, while the other became a chronic carrier for at least two months. No evidence was produced by this experiment to indicate that cloacal or bursal carriers are produced by vaccination against infectious laryngotracheitis, but laryngotracheal carriers may result from natural attacks of the disease. None of the vaccinated birds died, while four of the five controls inoculated intratracheally with the same virus died.

FIELD EXPERIMENTS

After the completion of the laboratory studies, which showed that vaccination against infectious laryngotracheitis is a possibility and that it may have some use on the poultry farm as a preventive, the following field experiments were carried out to determine its value under range conditions. Results are shown in Table 6.

Flock 1

This mixed flock of 1,402 birds was vaccinated on July 3, 1933, in seven and one-half hours. The birds were confined in the colony houses the night before. There were four helpers, two of whom caught the birds and passed them out while the other two held them for vaccination.

The vaccine was prepared by the modified Swift method eight months before use and stored in a Frigidaire. Immediately before vaccination the vaccine was ground up into a thin paste, using glycerine and saline as a diluent, in an unglazed mortar with an unglazed pestle.

Four days after vaccination the birds were examined for takes. At this time those birds showing poor takes or none were revaccinated from those presenting good and excellent takes.

This flock passed through the winter of 1933-1934 without infectious laryngotracheitis. This is the first time in four years that this disease has not appeared on this farm in the late fall or early winter and been responsible for considerable mortality and loss in egg production.

Flock 2

This flock consisted of 912 birds. The vaccine was prepared by the modified Swift method six months before vaccination, sealed in test tubes, and stored in a Frigidaire until carried to the farm. Vaccination commenced at noon, July 10, 1933, but had to be discontinued at 2:00 p. m. on account of rain. The job was finished at noon the next day, making a six-hour task. Four days after vaccination the birds were examined for takes.

Group 1 passed through the winter without incident. The percentage of takes in this group was 93.13. Group 2 had a slight outbreak of the disease soon after

moving into the laying houses in September. None of the birds died and the laying, which had just begun, was not noticeably affected. The percentage of takes in this group was 83.43, and as near as could be determined about 10 percent of the birds were affected. Since 10 percent of the vaccinated birds had either poor or no visible takes, it is reasonable to assume that as immunity wore off in this group, the susceptible birds contracted the disease from carriers, thus completing their protection against infectious laryngotracheitis.

A small flock of White Leghorns which was overlooked at the time of vaccination contracted the disease soon after being moved into the laying houses and about half of them died as a result of exposure to the vaccinated birds, although the latter showed no symptoms of disease at this time. This indicates that carriers were present in the vaccinated flock, and the White Leghorns contracted the disease from them.

Flock 3

This flock consisting of 450 pullets and cockerels was vaccinated in the forenoon of July 19, 1933. The vaccine was prepared according to the modified Swift method the day before vaccination. The birds in this flock were examined for takes five days after vaccination. The percentage of takes following the first vaccination were so high that second treatment was considered unnecessary by the owner. A cockerel in which no visible take was evident after vaccination died of infectious laryngotracheitis. The rest of the birds came through the year without showing any indications of the disease.

Flock 4

This flock consisted of 4,040 birds. Since the laboratory was not equipped to prepare vaccine for so many birds, it was decided to vary the method in order to meet the situation. Only enough vaccine was prepared in the laboratory to vaccinate 50 birds. The only object in preparing vaccine by the modified Swift method is to preserve it for use at a later time and for transportation. The treatment does not add anything to its properties; as a matter of fact such vaccines may be entirely unsatisfactory due to faulty desiccation. Theoretically, fresh vaccines prepared on the farm should be more virulent, insure a higher percentage of takes, and eliminate all possibility of poor vaccine, because if the virulence of the vaccine should prove to be unsatisfactory in the inoculated birds, it would not be used by anybody having practical experience with this method of producing immunity.

Accordingly 50 pullets and cockerels were culled from Flock 4 and confined in a colony house some distance from all other birds. These birds were inoculated intratracheally with the desiccated virus September 7, 1933. On September 11 the owner telephoned that the inoculated birds were coming down with the disease and judging by the severity of the symptoms the virus was satisfactory for vaccine. Hence it was agreed to begin vaccinating the next day.

On September 12 a dozen of the most marked clinical cases were selected from the inoculated flock, six in the morning and six in the afternoon, and fresh liquid vaccine prepared in the usual manner from the tracheal exudate. On this day 1,021 birds were vaccinated. The next day 1,535 birds were vaccinated using

TABLE 6. RESULTS OF THE VACCINATION OF FLOCKS IN THE FIELD.

Group	Number of Birds	Age in Weeks	Number of Takes				Total Percentage of Takes
			First Vaccination		Second Vaccination		
			+	0	+	0	
			Flock 1				
1	12	2	9	3	1	2	80.33
2	62	6	56	6	4	2	95.15
3	400	12	364	36	18	18	95.50
4	400	16	375	25	10	15	96.25
5	528	22	490	38	13	25	95.26
Total	1,402		1,294	108	46	62	95.58
			Flock 2				
1	481	10	442	39	6	33	93.13
2	431	20	347	84	14	70	83.73
Total	912		789	123	20	103	88.71
			Flock 3				
1	150	9	148	2	—	—	98.66
2	150	12	149	1	—	—	99.33
3	150	17	150	0	—	—	100.00
Total	450		447	3	—	—	99.33
			Flock 4				
1	1,021	16	1,008	13	3	10	99.02
2	1,535	18	1,520	15	4	11	98.63
3	284	18	279	5	1	4	98.59
4	1,200	20	1,167	33	26	7	99.42
Total	4,040		3,974	66	34	32	99.21
			Flock 6				
1	1,200	32	1,040	160*	3**	157	86.92
2	1,200	29	1,195	5	0	5	99.58
3	1,200	24	1,192	8	0	8	99.33
Total	3,600		3,427	173	3	170	95.27
			Flock 7				
1	800	20	768	32	—	—	96.00

*Of these birds 30 died as the disease had appeared two days before vaccination in two pens.
**Takes for the second vaccination were estimated, since only part of the birds were examined.

the exudate from 12 infected pullets and cockerels. On the 14th, 284 birds were vaccinated before the work had to be given up on account of rain. The vaccine left over from the birds that had been slaughtered was used to inoculate 12 more culls in order to insure a fresh supply of virus for use later. The rain continued until the afternoon of the 17th. The next day was fair and the remaining 1,200 birds were vaccinated with fresh liquid vaccine prepared from the last lot of inoculated culls.

During the three rainy days the unvaccinated birds were in a field about 50 yards from the last lot of vaccinated pullets. Largely on account of the rain the birds stayed in the colony houses most of the time. One colony house of 125 cockerels was more active than the pullets, and it was noticed that some of these cockerels were showing symptoms of infectious laryngotracheitis on the day the vaccination was recommenced. Some of these cockerels had natural outbreaks of infectious laryngotracheitis and some died. The other birds came through the year without incident.

Flock 5

This flock was not vaccinated, but it is closely related to the field experiments, and demonstrated far more conclusively than any set laboratory experiment possibly could the existence and dangers of carriers in vaccinated flocks. The history of this flock is somewhat vague and indefinite because records were not made at the time developments occurred, but it is sufficient to indicate the points suggested.

Flock 5 was visited February 9, 1934. Many of the hens showed active symptoms of infectious laryngotracheitis and some were dying. The caretaker reported that he was carrying out 15 or 20 dead hens per day. The roosters had swollen wattles and combs, but this appeared to be due to freezing and secondary infection. Cholera was excluded from the diagnosis by bacteriological examination of both roosters and hens. Two of the roosters were sick from the absorption of toxins from the edematous wattles, and one was reported as having died. The two sick roosters had been taking severe punishment from the others and were in bad shape. However they recovered in 24 hours after being removed from the flock and having the combs and wattles lanced.

The first lot of roosters had been purchased December 24, 1933, and the next lot two weeks later, or January 6, 1934. Infectious laryngotracheitis appeared soon after the second lot of roosters was added to the flock, although the exact date of the beginning of this outbreak is unknown. The pullets, 500 in number, had been purchased from a flock that has never had infectious laryngotracheitis. The evidence was against the roosters. Consequently the 64 living roosters were tested for carriers by inoculating exudate from the larynx and trachea into the larynx and trachea of susceptible chickens. Two susceptible chickens were inoculated from each rooster. This made a total of 128 chickens on the test. Of these chickens 124 remained healthy for 10 days, and four died from two of the roosters, one on the fourth day, two on the fifth, and one on the sixth. The test was repeated two weeks later with the same results, except that all four of the chickens were found dead on the fourth day.

The results of this experiment indicate that two chronic carriers of infectious laryngotracheitis were sold into a healthy flock as a result of the purchase of vaccinated birds. Of course it may be argued that this might have happened

anyway, because for a number of years the flock from which the roosters were purchased had had an outbreak of infectious laryngotracheitis every fall, which lasted nearly all winter, and the disease was first introduced into this flock by the purchase of breeding cockerels, before vaccination against this disease was known.

It is evident that adult birds from vaccinated flocks and from flocks having natural outbreaks of infectious laryngotracheitis should not be placed in flocks free of this disease, because of the danger of carriers. Unfortunately no satisfactory way of detecting chronic carriers of infectious laryngotracheitis is known at present.

Flock 6

The owner of this flock had attempted the eradication and control plan, but apparently the premises were not properly disinfected and the disease broke out almost simultaneously in two different pens after about two-thirds of the pullets had been moved in from the range. No vaccine was available to treat the flock, so it was decided to attempt to stop the disease by making up the vaccine on the farm from the virus occurring naturally in the outbreak.

It took four men three days to vaccinate this flock of 3,600 birds at the rate of 1,200 per day, working eight hours a day.

Other observations, besides those recorded in Table 6, were made on the vaccinated birds, as follows:

(1) 3,100 or 86.11 percent showed takes and no symptoms of disease.

(2) 330 or 9.16 percent showed both takes and symptoms of infectious laryngotracheitis, and 10 or 3.03 percent of them died.

(3) 134 or 3.74 percent showed no takes and no symptoms of disease.

(4) 36 or 1.00 percent showed no takes and symptoms of disease, and 20 or 55.55 percent died.

Flock 7

August 7, 1933, the writer visited a poultry farm on which an outbreak of infectious laryngotracheitis had just begun. This poultryman had started in the business the year before by purchasing pullets and cockerels from breeders and bringing them together in a single house. Infectious laryngotracheitis broke out soon after the birds were housed and lasted all winter, so that the owner decided to slaughter the birds for local trade.

A new house was built about 10 feet from the one in which some of the old birds still remaining on the farm were kept, and the first lot of pullets which had begun to lay had been placed in it 10 days before developing an outbreak of infectious laryngotracheitis. In the meantime, the disease appeared in some of the chickens on the range, which was about 20 feet from the new poultry house. There were approximately 800 chickens on the range.

It was decided to vaccinate the chickens using vaccine prepared from the pullets which had contracted the disease first and were hopelessly lost, since all of them were sick, a few had died, and about half of those remaining probably would die in the next three or four days. Unfortunately the writer did not have his vaccination outfit with him as the trip was taken for an entirely different purpose, and it was necessary to use improvised equipment. The 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, with results recorded in Table 6.

In addition it was estimated at the time the birds were examined for takes that:

(1) 704 or 88 percent showed takes and no symptoms of disease.
(2) 64 or 8 percent showed both takes and symptoms of disease.
(3) 28 or 3.5 percent showed no takes and no symptoms of disease.
(4) 4 or 0.5 percent showed no takes and symptoms of disease.

All of the birds recovered and remained free of infectious laryngotracheitis through the year except the four which showed no takes and symptoms of disease. The birds not showing takes were not revaccinated at the time the examination for takes was made and the results were entirely satisfactory to the poultryman.

Cloacal and Bursal Carriers in Field Experiments

After the field vaccinations had been completed it was felt that the presence or absence of cloacal and bursal carriers in vaccinated flocks was still unsettled, because the studies hitherto had been limited to one group of 55 birds (Gibbs, 1933b) and another group of 30, which may not have been sufficient to give a correct interpretation. So 620 birds from Flock 4 were systematically examined for cloacal and bursal carriers beginning two months after vaccination. This flock was divided into four groups of 155 birds. Each group was swabbed in the cloaca and bursa, and chickens inoculated intratracheally with the exudate until all of the birds had been examined.

The cloacas and bursas of 560 hens and 40 roosters were found to be free of microorganisms or viruses pathogenic to baby chicks. The chickens inoculated from these hens and roosters remained healthy throughout the experiment. The cloacas and bursas of 20 of the hens may not have been free of microorganisms or viruses, because the chickens inoculated from them developed dyspnea and died of asphyxiation as in infecticus laryngotracheitis. After death the larynx and trachea were found plugged with pseudomembrane. Histological studies of the larynx and trachea of the diseased chicks indicated that the inflammation was a desquamative epithelial inflammation. The disease could not be transmitted beyond the first group of chickens inoculated directly from the hens. No bacteria other than those occurring normally in the larynx and trachea could be found. The disease was not obtained by swabbing the tracheas of chickens with clean swabs. It occurred in the same proportion in a smaller number of unvaccinated hens. Therefore the disease did not appear to be traumatic or confined to vaccinated birds. The specific cause was not determined, although it occurred quite regularly in the same birds, as the following study shows.

Four groups of chickens were inoculated at different intervals with exudate from 10 vaccinated and 10 unvaccinated birds showing the disease, and from 10 vaccinated and 10 unvaccinated birds not showing the disease. Results are shown in Table 7.

A study of Table 7 shows that the disease was more persistent in the unvaccinated birds, but evidence was lacking to indicate that vaccination had anything

to do with it. The disease was probably due to a bacterium or virus which grew in the cloaca of the fowl but did not grow on the culture media used in this study, and either did not grow or did not maintain its virulence in the respiratory tract of the chickens beyond the first inoculation.

TABLE 7. INOCULATION EXPERIMENTS IN AN UNUSUAL DISEASE OF THE CLOACA AND BURSA OF FABRICIUS OF THE DOMESTIC FOWL.

Intervals in Weeks	Hens Showing the Disease		Hens Not Showing the Disease	
	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated
1	10	10	10	10
2	10	10	9	10
3	9	10	9	10
4	10	10	9	10
Total	39	40	37	40

Since the cloaca and bursa of Fabricius undergo rather radical changes in the growth of the chicken, it is anticipated from this and preceding studies that cloacal and bursal carriers of infectious laryngotracheitis may not exist for any great length of time. As the chicken grows, the mucous membrane of the cloaca is subject to the passage of excretions in both sexes, and the laying of eggs in pullets. These functions tend to thicken the mucous membrane and make it firmer and more resistant to mechanical injury, as well as to the attacks of micro-organisms and viruses. At five or six months of age, the bursa of Fabricius undergoes involution in the course of which this organ disappears entirely and is replaced by a bursal fold. The mucous membrane of the bursal fold is firmer in consistency and more resistant to the virus of infectious laryngotracheitis than that of the bursa of Fabricius.

DISEASES SOMETIMES MISTAKEN FOR INFECTIOUS LARYNGOTRACHEITIS

During the course of four years and a half of field investigations, representative cases from 131 epizootics have been studied, and some diseases simulating infectious laryngotracheitis clinically have been found. Laboratory studies revealed that these diseases are etiologically and immunologically different. Infectious laryngotracheitis was not only the most important from a mortality point of view, but it appeared to be the most common, as the following summary shows:

Coryza and colds	14
Conjunctivitis	5
Rhinosinusitis	3
Infectious bronchitis	2
Chickenpox and roup	4
Infectious laryngotracheitis	103
Total	131

These diseases were maintained in healthy birds in the laboratory either by contact or by inoculation with the respective exudates. In making the etiological studies, exudates were streaked on plates containing chicken infusion and chicken blood agar, and incubated aerobically, anaerobically, and in an atmosphere of CO_2, or sealed as Nelson (1933) found suitable for cultivating the causative agent in coryza of chickens, and as Delaplane and Stuart (1934), Schalm and Beach (1934), and Eliot and Lewis (1934) found efficacious for isolating an organism involved in purulent conjunctivitis, rhinosinusitis, and colds of chickens. Immunological studies were made on birds recovering from natural attacks of the respective diseases. The diagnostic differentiation of these diseases is especially important because of the bearing it has on vaccination against infectious laryngotracheitis.

Coryza and Colds

A gram negative hemophilic bacterium resembling those described by the investigators just mentioned was found in 8 of the 14 diseases classified as coryza and colds. The causative agent in this disease did not appear to be filtrable. Berkefeld V, N, W, and Seitz filters were used according to the technique of Ward (1928), Tang (1932), and Gibbs (1933b). All of the filters used in this study were tested and graded according to the technique of Ward and Tang (1929) and Krueger and Riter (1930).

The hemophilic bacterium did not appear to be as virulent in chickens reared in the laboratory as in field cases. Other factors such as exposure to cold, damp weather and improper feeding may have been responsible for these differences. Before this microorganism is accepted as the primary cause of coryza and colds in the domestic fowl, more intensive investigations of natural outbreaks are in order.

Fresh exudate from the nostrils and sinuses of chickens suffering from coryza or colds was swabbed into the bursa of Fabricius and produced mild inflammation of this organ as shown in birds necropsied five days after inoculation. Freshly isolated cultures of *Hemophilus gallinarum* induced a similar affection, while old laboratory cultures seemed to be quite innocuous. Chickens in which takes were evident retained their resistance to this disease for one month. Hens and roosters, or birds in which the bursa of Fabricius had disappeared, were refractory to this treatment.

Conjunctivitis

Five outbreaks of conjunctivitis in chickens six weeks or less in age were found, after several transfers in susceptible chicks, to be chronic eye forms of infectious laryngotracheitis. The poultrymen called this condition "greasy head" because the chickens frequently rubbed their heads on their shoulders, and the head, neck, and shoulders became smeared with exudate from the eyes, producing a very unsightly appearance.

Rhinosinusitis

Three outbreaks of rhinosinusitis occurring in Bristol and Plymouth Counties were found upon etiological studies to be due to mixed infections. One outbreak appeared to be due to a combination of coryza and infectious laryngotracheitis,

since *Hemophilus gallinarum* (Eliot and Lewis, 1934) was isolated in cultures and a virus indistinguishable from that producing infectious laryngotracheitis in susceptible birds after filtration with Berkefeld V filters. *Hemophilus gallinarum* and *Streptococcus bronchitis* (Gibbs, 1933a) were isolated from affected birds in the other two outbreaks, and the disease reproduced in healthy laboratory stock by instilling mixed suspensions of these microorganisms into the eyes and nostrils.

Preliminary experiments indicate that rhinosinusitis may be controlled by autogenous vaccination, using the technique recommended for autogenous vaccination in infectious laryngotracheitis.

Infectious Bronchitis

Two outbreaks of infectious bronchitis have been identified. One was in a flock of brooder chickens and the other was in a flock of roosters which had been housed by themselves. The disease did not appear to be especially serious in either the chickens or adult birds, and finally disappeared without treatment. Since infectious bronchitis appears to be relatively unimportant, and the location of the symptoms in the bronchi and bronchioles is rather inaccessible, no curative treatment is suggested for adult birds. However, the brooder should be thoroughly cleaned before being used again when the disease appears in chickens.

Chickenpox and Roup

Four outbreaks of chickenpox complicated with roup appeared in this study of field cases. These epizootics were in small chickens and rather difficult to diagnose at first sight. However, upon close examination characteristic pustules were found either on the edges of the beak or on the comb of some of the chickens, and satisfactory diagnosis made. Since chickenpox is a cutaneous disease and may be controlled by specific vaccination, these cases were not extensively studied.

WHEN AND HOW TO VACCINATE FOR INFECTIOUS LARYNGOTRACHEITIS

The Massachusetts Plan for the Eradication and Control of Infectious Laryngotracheitis (Gibbs, 1933c) is the most hopeful for the industry as a whole, because it will eliminate not only infectious laryngotracheitis but all contact diseases. This plan will never succeed unless the poultry associations get back of it and put it across. There is no money in this plan for commercial firms as there is in vaccines. Therefore commercial concerns will not put this plan into operation, but some of them will be led into the manufacture and sale of vaccines. There is undoubtedly a place for infectious laryngotracheitis vaccines for they enable the poultryman, so unfortunate as to have an infected flock, to save his birds until a more favorable time is reached for the complete eradication and control of the disease by sanitary methods.

Also it has been found (Gibbs, 1931b) that infectious laryngotracheitis is spread by both acute cases and chronic carriers, and vaccination does not eliminate the possibility of the spread of the disease from either of these sources, because vaccination is nothing more than the inoculation of the mucous membrane of the cloaca and bursa of Fabricius with the living virus and the symptoms and lesions are confined to these organs rather than the larynx and trachea. In

s birds die of asphyxiation due to the plugging of the larynx and vaccinated birds do not die because the larynx and trachea e birds have the disease and immunity is developed the same

It should not be forgotten that as long as acute cases in on the premises there is always danger of the spread ble birds.

vhich vaccination may reasonably be expected to be First, the virus from which the vaccine is made must ated by the way the disease attacks the birds sacrificed ways kill some of them in three or four days after Second, it is no use to vaccinate birds already sick with is. If the disease is pretty well scattered through the icate that it is useless to vaccinate. Also, birds badly a run-down condition generally are poor risks for *ircumstances should a flock be vaccinated against in- less the disease is already present on the farm and there spreading to susceptible birds.*

Preparation of Vaccine

cinate chickens on the range, laboratory vaccine may lt to prepare infectious laryngotracheitis vaccine in cale, and at the same time maintain a satisfactory batch, it is well to test such vaccines before use. late some birds from the main flock and inoculate e vaccine. If most of the birds come down with hin three days and some of them die, the virulence nay be used. But if the vaccine should prove to uld be destroyed and the premises thoroughly ial care not to let the disease reach the main inating against infectious laryngotracheitis f an experienced worker.

dy appeared in a small portion of the unization, then autogenous vaccine pared as follows: Take a bird ay the dead bird on a slit open the skin -bone. Care- as much of cutting ife,

some planning in order to make vaccine and birds to be vaccinated

Poultrymen should not indulge in the practice of borrowin
gotracheitis exudates from each other, for such practice will
of diseases other than infectious laryngotracheitis, and m
of the poultry industry in Massachusetts.

Vaccination

If the birds are housed they should be driven into a
crates standing on end, or some other fence. On th
convenient to fasten the required number of birds for a
houses the night before. In the case of laying hens, so
lighting facilities prefer to have the vaccinating done a
only necessary to lift the birds from the roosts with
in production as a result of handling.

A vaccinating crew ordinarily consists of three men:
to hold the birds, and one to do the vaccinating. T
with the birds, the other two should go beyond the r
light is good. The prepared vaccine should be on a t
Also some cotton should be twisted on the ends of some
the size of matches or larger.

The first man catches a bird and hands it to the
by the legs with one hand and the wings with the otl
and back to the third man, ready for vaccination
third man grasps the upper or dorsal fold of the
forefinger, and opens the cloaca. On the upper
pink groove will be seen leading into a little fold
he picks up an applicator, dips the cotton int
this groove, going clear into the fold or slit as
the bursal groove, the fold is the bursal fold
bursa of Fabricius. All of these parts mu
order to vaccinate properly. As soon a
bursal groove, the bursal fold, or the
plete and the bird should be p
group.

It should be point
hens and breedir
pullets and c
cavity, th
The

plugged with pseudomembrane just as the larynx and trachea are filled in birds sick or dead of infectious laryngotracheitis.

Vaccination is a medical treatment and medical treatments have their limitations. If the limitations 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.

SUMMARY AND CONCLUSIONS

1. The success of vaccination against infectious laryngotracheitis depends on the number of takes. The most favorable time for reading takes was found to be the fourth and fifth days after vaccination. Takes may be classified, according to degree of inflammation, as poor, fair, good, or excellent. Occasionally takes occur in the bursa of Fabricius which are not visible at the time of examining the live birds.

2. It has been determined that 94 percent takes in chickens one to three months of age, 97 percent takes in pullets and cockerels four to seven months old, and 90 percent takes in hens and roosters eight to twelve months of age insure a satisfactory degree of immunity for a flock as a whole. The takes should be good, fair, or excellent, for the immunity tends to wear off in birds showing poor takes. When vaccinating is once started, it should be pushed on to completion with as little delay as possible in order to avoid outbreaks of the disease.

3. After this laboratory information had been obtained, field vaccination was successfully accomplished in six flocks, or 11,204 birds.

4. A study of diseases simulating infectious laryngotracheitis was made for differential diagnostic purposes, and it was found that autogenous vaccines were specific for infectious laryngotracheitis, coryza, and rhinosinusitis, but not for each other. Therefore, it cannot be emphasized too strongly that before vaccination is resorted to for the control of infectious laryngotracheitis, a correct diagnosis of the disease or diseases infecting the flock is necessary for success.

5. Since laboratory vaccines for infectious laryngotracheitis have not been entirely successful in the hands of poultrymen, autogenous vaccines may have some use because of their specificity and availability at the time of greatest need. A method of preparing and using autogenous vaccines in infectious laryngotracheitis is outlined. It should be understood that this method of vaccination will save the flock if properly applied, but it cannot be depended upon to eliminate carriers; and for the good of the poultry industry as a whole it should be followed by the complete eradication and sanitary control of the disease. Vaccination for infectious laryngotracheitis merely enables the poultryman to choose the time for disposing of his birds and cleaning and disinfecting the premises occupied by them. When accepted in this light, vaccination is a valuable contribution to the control of infectious laryngotracheitis.

REFERENCES

1932. DeBlieck, L. A haemoglobinophilic bacterium as the cause of contagious catarrh of the fowl. Vet. Jour. 88 (1):9-13.

1933. Delaplane, J. P., H. O. Stuart, and H. Bunyea. A preliminary report of an apparently new respiratory disease of chickens. Jour. Amer. Vet. Med. Assoc. 82, n.s. 35 (5):772-774.

1934. Delaplane, J. P., and H. O. Stuart. Rhinitis—A respiratory disease in chickens. R. I. State College Bul. 29 (4):92-94.

1934. Eliot, C. P., and M. R. Lewis. A hemophilic bacterium as a cause of infectious coryza in the fowl. Jour. Amer. Vet. Med. Assoc. 84, n. s. 37 (6):878-888.

1931a. Gibbs, C. S. Saprophytic and secondary microorganisms occurring in the respiratory tracts of domestic fowls and chickens in health and disease. Jour. Bact. 29 (2):97-109.

1931b. Gibbs, C. S. Infectious laryngotracheitis carriers. Mass. Agr. Expt. Sta. Bul. 278.

1933a. Gibbs, C. S. Bronchitis of baby chicks. Poultry Sci. 12 (1):46-48.

1933b. Gibbs, C. S. The immunology of infectious laryngotracheitis. Mass. Agr. Expt. Sta. Bul. 295.

1933c. Gibbs, C. S. The Massachusetts plan for the eradication and control of infectious laryngotracheitis. Jour. Amer. Vet. Med. Assoc. 83, n. s. 36 (2):214-217.

1930. Krueger, A. P., and R. C. Ritter. The preparation of a graded series of ultrafilters and measurement of their pore size. Jour. Gen. Physiol. 13:409-419.

1933. Nelson, J. B. Studies on an uncomplicated coryza of the domestic fowl. Jour Expt. Med. 58 (3):289-304.

1934. Schalm, O. W., and J. R. Beach. The etiology of a respiratory disease of chickens. Science 79 (2053):416-417.

1932. Tang, F. F. Absorption experiments with the virus of vaccinia. Jour. Bact. 24 (2):133-143.

1928. Ward, H. K. Further notes on the filtration of the virus of vaccinia. Jour. Expt. Med. 50:31-40.

1929. Ward, H. K., and F. F. Tang. A note on the filtration of the virus of herpetic encephalitis and of vaccinia. Jour. Expt. Med. 49 (1):1-4.

PUBLICATION OF THIS DOCUMENT APPROVED BY COMMISSION ON ADMINISTRATION AND FINANCE
3M-8-34. No. 2112