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B584: Controlling Infectious Bronchitis in Maine Chickens

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Controlling
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in Maine
chickens



Bulletin 584
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This study was part of a northeastern regional project on poultry disease.

BULLETIN 584

CONTROLLING INFECTIOUS BRONCHITIS IN MAINE CHICKENS

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History of the Disease in Maine

Infectious bronchitis has been a respiratory problem in domestic chickens in the United States since its discovery in 1931.^{1*} The disease has also been found in Canada,² England,³ Netherlands,⁴ Italy⁵ and Japan.⁶

Witter⁷ and Reed⁸ reported in 1939 that "colds" in chickens were prevalent in some areas of Maine. In 1944 H. Van Roekel, Department of Veterinary Science, University of Massachusetts, found neutralizing antibodies in the blood of infected chickens. It was assumed up to this time that the outbreaks were sporadic and local in nature. During the fall and winter of 1944-45 the disease constituted the greatest disease hazard of laying flocks. A survey was made in Maine by questionnaire in March, 1945. From 724 replies it was found that 18 per cent reported respiratory disease outbreaks. Ninety-eight of these outbreaks were reported in flocks of laying birds of which 57 per cent indicated a marked decrease in egg production.

At a special meeting held in Hallowell, Maine, April 6, 1945, a vaccination program for control was evolved and approved by the directors of the Maine Poultry Improvement Association. After approval by the State Department of Agriculture this was put into immediate effect. Twenty-two flocks, representing 90,394 birds, were enrolled in the program in 1945. The number of flocks tripled the next year and has increased markedly during succeeding years. In 1948-49, 325 flocks comprising 657,930 birds and in 1949-50, 897 flocks comprising 1,386,647 birds were inoculated.

The program grew out of the request of poultrymen to obtain from the University of Maine the infectious bronchitis virus to inoculate their flocks. No commercial bronchitis vaccines were available at this time. A fieldman was sent to the farm when the birds were 6-12 weeks of age and 5 per cent of the birds were swabbed in the trachea with a diluted saline suspension of mucosal scrapings from an infected trachea. In the

* Numbers refer to literature citations on page 25.

beginning the pathogenic bronchitis virus was propagated in young live birds.

Early in 1950 the field strain of the infectious bronchitis virus was propagated in chicken embryos and the infected allantoic fluid was used for a vaccine.

These studies have been conducted with a so-called severe or pathogenic strain of the infectious bronchitis (IB) virus from 1945 to 1956.

In July, 1957 the University discontinued its policy of manufacturing infectious bronchitis vaccine. This was done because satisfactory commercial vaccines had become available and it seemed desirable for the institution to concentrate its efforts on diagnosis and research. During this 10-year period considerable information was obtained which is still of value in the control of this disease.

Newcastle disease was discovered in Maine in 1947 and an active wing-web vaccination program for this disease was soon put into effect. In spite of vaccination for infectious bronchitis and Newcastle disease, respiratory infections continued. Many problems such as the duration and degree of immunity in vaccinated flocks were posed.

This publication reports on the investigations of some of these respiratory disease problems and steps taken to improve the vaccines.

Preparation of University Vaccine

The original strain of infectious bronchitis virus was obtained from Dr. Henry Van Roekel, University of Massachusetts, Amherst, Massachusetts. This virus was passed through birds many times serially in the early studies and later was used in the chicken egg embryo studies and has been passed at least 60 times in them serially.

Until 1950 the vaccine was prepared by infecting 3-6 week-old susceptible birds. After infection was established clinically the tracheas were removed, the epithelial lining was scraped with a dull scalpel and the material obtained suspended in .85 per cent saline. Empirical methods were employed for standardization. The vaccine was diluted and preserved in brown bottles in 12 ml. amounts, frozen and after a trial on susceptible birds was used in the field by poultrymen.

Since 1950 the virus has been propagated in 10-day old chicken egg embryos via the allantoic sac route. The eggs were candled at 24 hours and the ones with dead embryos were discarded. At 48 hours the embryos were chilled in the refrigerator for two hours and the allantoic fluid harvested and stored in screw cap vials in a commercial deep-freezer at -10 to -20° F. Prior to deep-freezing all samples were subjected to a sterility check by taking one loopful of amniotic-allantoic (a.a.) fluid and placing it in a fluid sodium thioglycollate culture medium.

The effectiveness of the IB vaccines was evaluated by means of a standard report form which was sent with the vaccine to the poultrymen who returned the completed questionnaires to the laboratory. A sample form is shown in the appendix. The vaccine was sent to the field in a double cardboard carton containing a can of ice with the small bottle of vaccine in an insulated bag (figure 1). Occasionally poultrymen would pick up the vaccine at the laboratory and go directly to the field to vaccinate flocks.

Over the period of study more than ten million doses of vaccine were distributed throughout the state. In general favorable results were obtained. The protection afforded Maine's poultry industry through this service without question contributed benefits in financial returns and industry growth that should justify the services of the diagnostic laboratory and research program for years to come.

Method of Application

At the beginning of the program in 1945 the vaccine was applied by dipping cotton tipped applicator sticks in the thawed vaccine and swabbing the throats (tracheas) of five per cent of the flock. The disease would establish itself in these birds and due to the infectious nature of the disease would spread to the remainder of the flock.

In 1949 Lipman Poultry Co., Augusta, Maine, began using the vaccine by spraying it over the birds with an ordinary fly sprayer. Perhaps this method of application had been tried earlier but we have no official record of it. The vaccine was diluted by adding one part of vaccine to six parts of water. To the best of our knowledge, this was the first spray application of vaccines for mass immunization—a method to become popular later with commercial vaccines.

TABLE 1
Methods of Field Application of Bronchitis Vaccine 1949-1957

Period	Number of flocks reporting	Per cent of flocks treated by			
		Spray	Swab	Spray and swab	In water
1949-50	779	57.8	35.8	6.3	0.1
Sept. '51 to Oct. '52	518	44.9	45.3	9.4	0.001
Sept. '52 to Oct. '54	753	55.0	36.4	7.6	0.01
Sept. '54 to Feb. '56	976	59.3	33.6	5.9	0.01
March '56 to Sept. '57	369	59.9	32.6	7.1	0.003
All flocks 1949-57	3395	55.7	36.4	7.1	0.01



FIGURE 1. A shipment of infectious bronchitis vaccine being prepared to be sent into the field. Box in background is of double thickness cardboard, and the shipping bag within the box is insulated. Can at left is filled with ice in which the small vial of vaccine on top of the can will be packed. Cotton swabs are included in each shipment along with the report form in the foreground. The form is similar to the one reprinted on page 26.

By 1951 many poultrymen were using the vaccine by mixing it with water given to the birds to drink, another novel method to become commercialized later.

A summary of the various methods of application used during 1949 to 1957 is given in table 1. The spray treatment was used with 55.7 per cent of the flocks, the swab treatment with 36.4 per cent, and a combination of spray and swab for 7.1 per cent. The vaccine was added to the drinking water with 0.01 per cent of the flocks.

Field Reaction to Infectious Bronchitis Virus from the Seventh Chicken Embryo Passage Used as a Vaccine

This experiment was set up with the cooperation of 69 poultrymen whose flocks involved 108,604 replacement pullet birds. Twenty-eight flocks were vaccinated on range and 41 in chicken houses. Eleven flock owners reported the weather as unfavorable with rain, cold and damp or humid weather. The vaccination period covered was from February through September, 1951.

The vaccine was prepared as previously described and received by the poultryman as shown in figure 1. The methods of vaccination included 55 per cent of the flocks by spray application, 38 per cent by swab, 6 per cent by spray and swab and 1 per cent in the drinking water.

Total mortality during the course of the disease averaged .007 per cent for all methods. In 43 flocks the losses were zero, whereas an extreme mortality of 10 per cent was reported in one flock of 2,200 birds, and 6.0 and 6.5 per cent was reported in two flocks of 1,700 and 608 birds respectively.

The symptoms were judged by the poultrymen to be mild in 25 per cent of the flocks, average in 43 per cent, severe in 24 per cent and no symptoms in the remaining 8 per cent.

More details as a result of each method of application are shown in the following table.

TABLE 2
Symptoms Produced from Seventh Chicken Embryo Passage

Method of vaccination	No. of flocks*	Per cent of flocks showing symptoms of			
		Mild	Average	Severe	None
Spray	43	18.6	41.9	32.5	7.0
Swab	28	35.7	42.9	10.7	10.7
Water	1	—	100.0	—	—
All methods	72	25.0	43.0	23.6	8.4

* Column does not add to the total number of flocks because some growers used two methods.

The only other diseases reported by the poultrymen were cecal coccidiosis in one flock, intestinal coccidiosis in one flock and leucosis in two flocks.

Chicks from 3½ weeks of age up to 17 weeks were vaccinated. The duration of symptoms was from 7 to 14 days in 62 per cent of the flocks. No correlation was noted between the age at vaccination and duration of symptoms.

Rhode Island Reds, Barred Plymouth Rocks, White Rocks, New Hampshire Reds and RIR X BPR cross birds were vaccinated.

Combined Results of Field Application of University Bronchitis Vaccine from 1949 to 1957

Table 3 shows some of the results of the field application of the University bronchitis vaccine. It will be noted in the 9-year period covered by the table that the seventh serial embryo passage was the highest attained.

The most significant feature of this whole study is that, even with other causes of mortality being included, less than 1 per cent of chickens died from vaccination with infectious bronchitis virus. A total of 3,395 flocks totaling 6,209,850 birds were studied over the 9-year period. The average mortality for this period was 0.95 per cent.

Table 3 shows that there was no appreciable difference in mortality between the bird and embryo passages or from the early studies in 1949 to 1957. It is also evident that low serial embryo passage does not appear to alter the virus in any way.

In many of the trials the time of year covered both winter and summer so that it is difficult to relate cold or warm weather to the total mortality. In spite of the fact that perhaps some of the answers to

TABLE 3
Per Cent Mortality Reported in Using University Bronchitis Vaccine
Applied as Various Passages from 1949 to 1957

Vaccine passage	Period applied	No. of flocks reporting	Number of birds	Per cent mortality
3-37 Bird Passages	1949-50	779	1,327,639	.78
Emb Passages No. 2	Aug. '51 to April '52	55	73,554	3.34
No. 3	Dec. '51 to June '52	113	207,582	1.57
No. 3-2	May '52 to Sept. '52	195	270,371	5.40
No. 3 (new lot)	Sept. '52 to Feb. '54	333	602,495	0.91
No. 4	March '52 to Sept. '52	155	259,786	0.0056
No. 4-1	May '51 to Nov. '53	260	497,384	1.20
No. 7	Nov. '53 to April '56	386	666,848	0.67
No. 7	Aug. '54 to April '55	228	668,363	0.46
No. 7-3	June '54 to Sept. '54	160	272,716	0.40
No. 7-2	May '55 to Feb. '56	362	640,411	0.48
No. 7-3	March '56 to Aug. '57	369	720,701	0.56
All flocks	1949-1957	3395	6,209,850	0.95

questions covering the results of the vaccine could not be considered to be entirely valid the overall picture is significant due to the large numbers.

Regardless of the standardization obtained by commercial companies for vaccine production there is no doubt that the varied environmental conditions contribute to the variable results.

Severity of Symptoms

The results from a total of over 6 million birds in 3,395 flocks are reported. These results correlate very well with the previous study. About 48 per cent of the flocks had average symptoms, 16 severe, 24 mild and 12 per cent had no symptoms. The following table gives details of severity of symptoms with infectious bronchitis virus.

TABLE 4
Severity of Symptoms Developing from Field Application
of University Bronchitis Vaccine

Period	No. of flocks reporting	Per cent of flocks showing symptoms of			
		Mild	Average	Severe	None
1949-50	779	28.7	45.6	15.6	10.1
Sept. '51 to Oct. '52	518	20.7	48.2	20.3	10.8
Sept. '52 to Oct. '54	753	24.5	51.7	17.2	6.6
Sept. '54 to Feb. '56	976	24.5	53.2	11.0	11.3
March '56 to Sept. '57	369	19.2	43.9	14.6	22.3
All flocks	3395	23.5	48.5	15.7	12.3

Duration of Symptoms

Infectious bronchitis is a disease which spreads rapidly and has a rapid course. This is true if there are no other complicating respiratory diseases. Frequently IB produces a stress to aid the start of another disease. The following table shows that in the majority of cases the course of the disease from time of vaccination was 7 to 14 days.

From this study it is obvious that 65 per cent of the symptoms have subsided by the end of 14 days. It is important that the poultryman realize that IB alone is of short duration.

TABLE 5
Duration of Symptoms Resulting from Field Application
of University Bronchitis Vaccine

Period	No. of flocks reporting	Per cent of flocks with duration of symptoms in weeks			
		0-1	1-2	2-3	over 3
1949-50	779	21.4	44.8	20.5	13.3
Sept. '51 to Oct. '52	518	12.6	48.7	19.8	18.9
Sept. '52 to Oct. '54	753	12.7	50.0	24.4	12.9
Sept. '54 to Feb. '56	976	20.9	47.9	20.7	10.5
March '56 to Sept. '57	369	37.9	28.4	18.4	15.3
All flocks	3395	21.0	44.0	20.8	14.2

Incidence of Infectious Bronchitis in Poultry in Maine

Many poultrymen have questioned the value of vaccinating, suggesting at times that the incidence of the disease is low and at other times that the incidence is so high that the birds will get the disease anyway.

Table 6 shows the incidence of infection during 1956 in 121 flocks that were not vaccinated. The incidence of 85 per cent in non-vaccinated flocks shows that the disease is very common. There were, however, a few flocks sufficiently isolated so they did not experience natural field outbreaks.

The significance of these figures is that the disease in its natural form is almost inevitable. Therefore, vaccination seems to be a logical procedure for preventing bronchitis. Vaccination would then prevent broilers coming down with this disease a week prior to marketing or

TABLE 6
Serological Incidence of Infectious Bronchitis
in Non-vaccinated Maine Chickens in 1956

Age in weeks	Number of flocks	Number of birds tested	Per cent of flocks tested	
			Positive (had disease)	Negative (no disease)
Less than 5	3	11	100.0	—
5	7	25	71.4	28.6
6	5	18	100.0	—
7	7	20	57.1	42.9
8	9	29	77.8	22.2
9	11	39	90.9	9.1
10	17	56	94.1	5.9
11-14	33	109	81.8	18.2
15-20	14	49	85.7	14.3
More than 20	15	46	93.3	6.7
All flocks	121	402	85.1	14.9

layers getting the disease just prior to or during egg production. Both situations would cause severe financial loss to the poultrymen.

For many years the University of Maine has been recommending that poultrymen vaccinate their young pullets with infectious bronchitis virus in order to avoid financial losses due to this disease striking when the birds were in high egg production. This program has been criticized by some, especially since information has been published from other sources indicating that this disease in pullets causes permanent injury to the reproductive tract. The University Department of Poultry Husbandry laying flock has been vaccinated for at least ten years and there is no evidence to date of lowered egg production. In fact production has increased over the years.

Effect of Vaccination on Hatchability

A survey was conducted in 1955 on the hatchability of flocks within the state of Maine. A wide range of figures was obtained due to different breeds, seasons and general environment.

Nearly two thirds of the flocks reporting were vaccinated with the University vaccine applied as a spray, one fourth of the flocks received an intranasal or other type of vaccine, only one tenth of the flocks were not vaccinated.

The average hatchability for all flocks was 74 per cent and varied from a low of 68 per cent to a high of 81 per cent. Other data show that hatchability has increased in recent years. These studies indicate that infectious bronchitis vaccine has not been detrimental to egg production or hatchability.

Duration and Degree of Immunity of Chickens Vaccinated at 5 to 9 Weeks of Age, Measured by Serology and Challenge

For several years some poultrymen had stated that, although the laying stock had been bronchitis vaccinated the previous season, when the young chicks were vaccinated the older birds came down with a respiratory disease. It was, therefore, decided to design an experiment to determine the duration and degree of immunity induced by IB vaccination.

Four hundred New Hampshire chicks from the University of Maine flocks were sprayed with IB vaccine in the brooder house May 18, 1951. These chicks were in four lots of 100 each and were 5, 6, 8 and 9 weeks of age respectively when vaccinated. After visible symptoms in two days and a normal reaction period and uneventful recovery, the chicks were placed in range shelters and reared to laying age under usual poultry

husbandry practices. Each age group was identified by colored leg bands and all groups were housed together in the laying house.

The objective was to test each age group (four birds from each group) at monthly intervals with complete serum neutralization tests to determine their immunity. The birds were also challenged by taking two birds to a separate brooder house and swabbing the trachea with a swab soaked in infectious bronchitis virus.

Starting at ten weeks after vaccination, serum was tested from four birds from each of the four age groups every month. There was considerable variation between individual birds but at no time did any bird ever drop below 10^{+3} neutralizing doses. A figure of 10^{+2} or higher is considered immune. Some birds had a very high neutralizing index. Three-fourths of the birds tested had 10^{+6} neutralizing doses per unit of serum. The following graph shows the average immunity response to IB vaccination during nearly one year after vaccination (figure 2).

The results of the IB virus challenge were as follows:

First Challenge—six months after vaccination the birds showed mild coughing but IB virus was not isolated by the chicken egg embryo technique. A CRD agent which produced a turkey sinusitis was isolated in turkey sinuses.

Second Challenge—There were no symptoms on Jan. 28, eight months after vaccination.

Third Challenge—There were no symptoms on May 6, twelve months after vaccination.

Fourth Challenge—There were no symptoms evident on July 8, fourteen months after vaccination.

These data⁹ clearly indicate that the vaccinated birds in all age groups were solidly immune to infectious bronchitis for as long as 58 weeks after vaccination.

A statistical analysis of the data justified the following conclusions.⁹

1. The age at which the birds were vaccinated (5, 6, 8 or 9 weeks of age) had no significant effect on immunity as measured by the serum neutralization (SN) test.

2. The virus titer (the IB virus used in the SN test) had a highly significant effect on the measurement of immunity as determined by the SN test. Therefore, every laboratory should use a high titered SN, IB virus to accurately measure immunity.

Duration and Degree of Parental Immunity to Infectious Bronchitis Virus of Day-old Chicks from Vaccinated Hens

Due to the fact that almost 100 per cent of replacement laying flocks in Maine are IB vaccinated it is important to determine the degree of

immunity passed on to the chicks. This information is of importance to determine the best age to vaccinate in order to induce life-long immunity. These data are important today because they reveal why a variation is to be expected in vaccinated chicks.

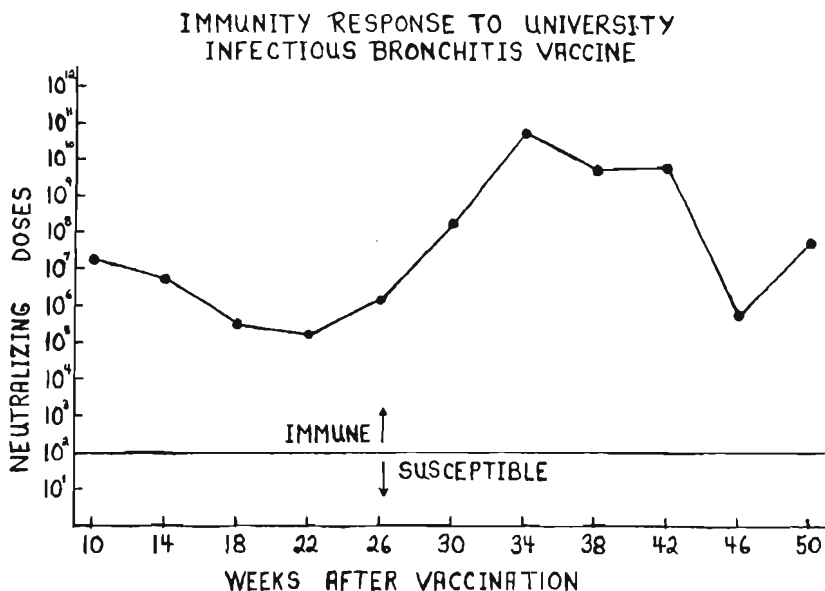


FIGURE 2. The average immunity response to infectious bronchitis vaccination during nearly one year following vaccination.

On May 20, 1952, approximately 200 chicks were hatched from the immune hens used in the previous experiment. It was assumed these chicks would have a high degree of parental immunity to infectious bronchitis. Two hundred chicks also were hatched from susceptible hens and used for controls.

The chicks were bled at weekly intervals for five successive weeks to determine how long the parental immunity remained in the chick. The birds were placed in isolation quarters and were wing-banded so that the parentally immune and susceptible chicks could be detected. This enabled the investigator to follow an individual bird which might be bled on several occasions.

The results are shown in table 7 for 284 parentally immune chicks and for 72 that were parentally susceptible. In evaluating the data from these chicks a neutralizing dose of 10^{+1} or less was considered susceptible, 10^{+2} as marginal, and 10^{+3} or greater as immune. Parental immunity was no longer present in chicks after 21 days. Up to seven days nearly all chicks were immune, but at 14 days about 50 per cent had

dropped below $10+^3$ ND, and by 21 days 90 per cent showed little evidence of immunity to IBV. In the controls, only three chicks were immune out of 10 tested at one day of age. None of the control chicks showed immunity after seven or more days.

At 15 days of age three chicks from the susceptible and three from the parentally immune group were placed in separate cages and each swabbed in the trachea with a third-embryo passage of IBV for a challenge (usual method). The susceptible birds began sneezing at five days and the parentally immune in six days. Symptoms were not severe and the birds soon recovered.

The chicks (parentally immune and susceptible) that were placed in a brooder house showed evidence of a respiratory disease at 15 days of age. An examination of the wing bands of the chicks showed that those exhibiting symptoms were the susceptible chicks. The immune chicks did not appear to cough and sneeze. This condition, which cleared up in 10 days, was assumed to be infectious bronchitis because of a positive SN test to IBV, although no virus was isolated.

From these studies it would seem that a large dose of virus was required to produce infection with IBV at two weeks of age. By three weeks of age it would appear that a small dose of IBV would produce the disease.

Table 8 presents the results of neutralizing doses of IBV in individual birds. This shows that at one day of age a chick would have a high SN titer, which by five weeks of age would decrease to zero.

TABLE 7

Duration of Immunity in Parentally Immune and Susceptible Chicks Based on Serological Results of SN Tests to IB

Age, in days	Number of chicks			All chick tests
	Susceptible ($10+^1$ or less)	Marginal ($10+^2$ ND)	Immune ($10+^3$ or higher)	
Parentally Immune:				
1	0	0	36	36
7	0	1	44	45
14	7	15	26	48
21	33	13	4	50
28	51	2	0	53
35	49	3	0	52
Total number	140	34	110	284
Parentally Susceptible:				
1	1	6	3	10
7	8	1	0	9
14	12	0	0	12
21	15	0	0	15
28	13	0	0	13
35	10	3	0	13
Total number	59	10	3	72

TABLE 8
Number of Neutralizing Doses to IB of Individual
Parentally Immune Chicks

Wing band number	Number of neutralizing doses for chicks bled at					
	1 day	7 days	14 days	21 days	28 days	35 days
610	10 ³	10 ⁴	10 ⁰	10 ⁰	10 ¹	10 ⁰
826	10 ⁶	10 ⁰	10 ⁷	10 ³	10 ²	10 ¹
829	10 ³	10 ⁵	10 ²	10 ¹	10 ¹	10 ⁰
883	10 ⁷	10 ⁵	10 ⁵	10 ²	10 ¹	10 ¹
586	10 ⁷	10 ⁶		10 ¹		
590	10 ⁶	10 ⁷	10 ⁵	10 ²		10 ⁰
621	10 ⁴	10 ⁶			10 ⁰	
804	10 ⁵	10 ⁴			10 ¹	10 ¹
805	10 ³	10 ⁵		10 ⁰		10 ²
815	10 ⁴	10 ⁵	10 ²	10 ¹		10 ⁰
840	10 ⁴	10 ⁶		10 ⁰		10 ⁰
841	10 ³	10 ⁶				10 ¹
844	10 ⁰	10 ⁵	10 ³		10 ¹	
Geometric mean	1,193,784	203,095	1,930	12.9	10.0	4.0

Results of Day-old Broiler Vaccination

Day-old broiler vaccination was conducted in the field using different dilutions of allantoic fluid as a vaccine. The vaccine was prepared as previously except that dilutions of 1:10 and 1:80 were used. The chicks were White Rock broilers varying in flock size from 4,000 to 12,000 birds. In the first studies, 16 flocks consisting of 125,000 day-old birds were sprayed in the chick boxes.

The minimum age of clinical signs following vaccination was four days of age. The minimum duration of take was 21 days. An attempt was made to analyze the data to see if vaccination was profitable on the basis of flocks the farmers had raised in the previous year. So much variation existed between management practices, feeding, genetics, etc., that the material could not be analyzed. However, no undesirable reaction was noted.

Graphs I and II (figures 3 and 4) represent the mortality for the first four weeks and for the entire growing period. From a total of 125,000 birds, the mortality during four weeks was 2.0 to 2.9 per cent in 52,000 birds. The mortality extended from 0.5 per cent in 5,000 birds to 4 to 4.1 per cent in 24,000 birds. The total mortality for the whole growing period was 3.1 to 4 per cent in 36,000 birds with variations as low as 1.0 per cent in one 5,000 flock and as high as 11.45 per cent in a 6,500 flock. Table 9 shows the mortality by weeks of the 16 different flocks for the first four weeks. This table indicates that the mortality was quite low for this period and was probably not increased by vaccination. On autopsy dead birds from each of the 16 flocks showed at least one other disease present and sometimes several.

Birds were tested from each flock at 1, 5, 8 and 10.5 weeks of age in order to study the immunity response. All flocks showed good parental immunity at one week of age. The dilution of vaccine had no effect on the immunity. The age at which birds were tested did not seem to influence the number of positive birds. A total of 174 birds were tested for immunity and of this number, 64 per cent were immune and 36 per cent susceptible.

The following tabulation shows the data relative to the number of immune or susceptible birds with each egg embryo passage:

Egg Embryo Passage

E.E. 7	E.E. 14	E.E. 18
37 Positive	27 Positive	49 Positive
37 Negative	16 Negative	9 Negative

After the completion of the above studies it was decided to continue field studies. The results of these studies are shown under the respective year in which the work was done.

In 1955 infectious bronchitis immunization with 50-51st egg passage, a strain of virus passed serially in embryos 50 times, was prepared as a vaccine and used as a spray on replacement pullet flocks.

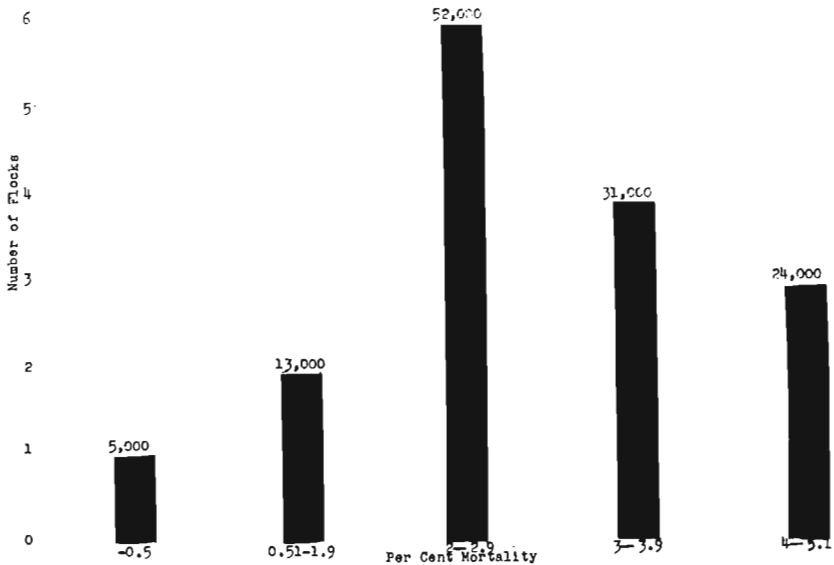


FIGURE 3. Day old bronchitis vaccination. The graph represents mortality from all causes for the first four weeks after vaccination. Sixteen flocks or 125,000 birds are included in this test.

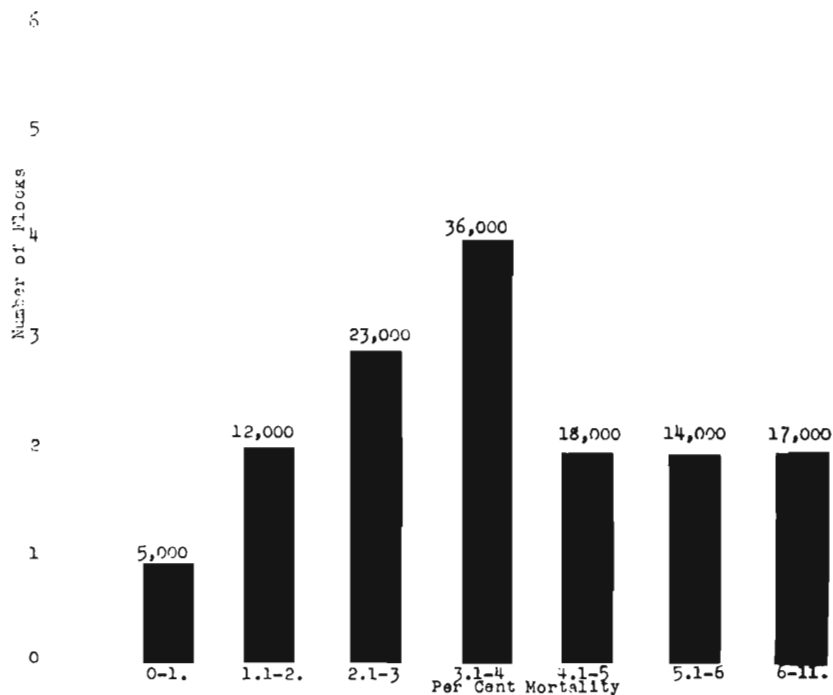


FIGURE 4. Day old bronchitis vaccination. The graph represents total mortality for the broiler growing period. Sixteen flocks or 125,000 birds are included in this test.

TABLE 9

Total Mortality by Flocks for First Four Weeks and at Market Age, for Day-old Vaccinated Chicks

Flock No.	No. birds	1st week	2nd week	3rd week	4th week	Total loss	Per cent loss first 4 weeks	Total mortality at market time
1.	8,000	62	28	20	6	116	1.45	4.30
2.	8,000	79	62	58	11	210	2.62	1.78
3.	8,000	180	155	42	29	406	5.07	5.09
4.	5,000	50	23	16	15	104	2.08	3.20
5.	6,500	42	63	89	63	257	3.95	11.45
6.	4,000	43	31	10	4	88	2.20	1.16
7.	8,000	113	58	48	12	231	2.88	3.14
8.	10,000	146	177	54	30	407	4.07	6.22
9.	10,000	101	108	17	18	244	2.44	3.64
10.	6,500	119	56	15	5	195	3.00	5.55
11.	6,000	117	49	47	36	249	4.15	5.71
12.	10,000	74	70	57	21	222	2.22	4.05
13.	8,000	150	62	27	17	256	3.20	3.10
14.	5,000	45	29	10	5	89	1.78	2.06
15.	10,500	110	108	87	35	340	3.23	8.48
16.	12,000	243	50	41	19	353	2.94	2.59

Nine pullet flocks vaccinated reacted with mild to average symptoms and no mortality and were immune to a serum-neutralization (SN) against the IBV. Favorable results prompted 200,000 doses to be used on broiler flocks with practically no signs following vaccination. SN tests were erratic and further study indicated parental immunity varied the results. Immunization with this high passaged strain of IBV indicates that immunity was produced with mild reactions and no mortality.

In 1956 a high passaged strain (50) of IBV was used as a vaccine on broilers in the field. The use of 150,000 doses has shown that approximately 70 per cent of the birds examined at ten weeks of age were serologically immune. The limiting factor in this spray type of vaccination is the CRD complex which occurs if birds are vaccinated older than two weeks of age.

In 1957 an IB vaccine of 51 passages was used on 246,500 birds from 12 flocks. This test was conducted under the most severe winter conditions. Although immunity was successfully established, the severe initial reaction and subsequent secondary effects which usually resulted indicated this strain of virus would not be entirely satisfactory unless the chicks were completely free of CRD. Chicks sprayed with a high passage IBV during the first seven days showed 70.9 per cent to be immune at 50 days of age and 68 per cent to be immune at 70 days of age.

Duration of Immunity Produced in Chicks Vaccinated When One Day Old

The objective of this study was to determine the duration of immunity at weekly intervals for chicks vaccinated when one day of age. A total of 464 chicks of various breeds (W.W., R.I.R., B.P.R., B.R. X R.I.R.) were obtained from the University Department of Poultry Husbandry on February 13, 1951. These chicks were wing banded and placed under a gas brooder. Chicks were sprayed while still in the boxes with the regular EE 7 infectious bronchitis vaccine diluted 1:20 at the rate of 70 ml. per 1000 and the covers replaced on the boxes for 20 minutes.

Four birds were bled by sterile technique at weekly intervals up to 16 weeks of age. The serum was deep-frozen at -10° F. and SN tests were completed at a later date.

Slight respiratory signs were noted four days after spraying, and on the fifth day these signs could be readily detected by holding the chick to one's ear and listening for rales and gurgling sounds. No serious symptoms were encountered and by 10 days of age all signs had disappeared. At 10 weeks of age all the birds were held under average crowded conditions, and no disease other than the experimental bronchitis disease

was noted at any time up to 16 weeks of age when the experiment was concluded.

When the birds were 16 weeks of age they were challenged by spraying with the regular EE 7, IB vaccine. Careful observation over a 4-week period did not reveal any evidence of respiratory disease.

A typical picture of parental immunity, indicated by an SN titer, decreased by the second week but increased tremendously by the fifth week indicating an acquired immunity. An immune status prevailed except for the 9th, 10th and 13th weeks of age. This drop could be accounted for by biological variations in the individual chicks selected. An immune response is not a uniform logarithmic rise but varies tremendously between individuals.

The spray challenge at 16 weeks of age did not produce a respiratory disease or affect the birds in any way. This together with the serological picture would indicate a high degree of immunity produced from the day-old vaccination. Beginning at five weeks of age, 43 birds were tested at intervals up to 16 weeks of age. Of this number 28 were positive or immune and 15 were negative or susceptible. This indicates a 65 per cent immunity from day-old vaccination.

It was concluded that vaccination of day-old chicks by means of a spray in the chick boxes was effective and produced immunity which lasted up to 16 weeks of age.

Infectivity of IBV from Chicken Egg Embryos at Different Serial Passages

This strain of virus was serially passed 50 times in chicken egg embryos. The results of these passages are shown in table 10. The only significant feature of these passages is that after the 20th embryo passage the mortality was above 50 per cent in 16 of the 30 passages. However, since the mortality never reached 100 per cent this would indicate that this virus was not lethal to all embryos and consequently the virus had not become completely adapted.

The infectivity of the virus, when made into a vaccine by the usual methods, was checked at the following passages with complete success which was indicated by producing symptoms of the disease and inducing immunity as measured by an SN test.

Passage	No. of birds treated All of which showed symptoms	Age
7th egg passage	108,604	Young adults
14th " "	47,500	Day-old
21st " "	32,500	"
25th " "	45,500	"
50th " "	17,950	Young adults

Another study was made to determine the infectivity of whole ground embryo material. Eggs were injected with seed virus in the usual manner. After the a.a. fluid was harvested, the top of the egg shell was peeled away and the yolk discarded. The embryo and chorioallantoic membrane were carefully moved to a large-mouth, screw-cap sterile jar. To 750 grams of material 750 ml. of buffered saline was added. This material was run through a colloid mill* of the horizontal type with an approximate speed of 8,000 r.p.m. The material was processed about 30 minutes down to a #28 size particle. The material was treated as straight a.a. fluid and a vaccine prepared in the usual manner. The embryos were not ground to a smaller size because an increase in temperature during further processing might have killed the virus.

One lot of the colloid material was used as a day-old spray. The flocks were 6,000 to 9,500 in size. A favorable take† was observed in four days and three SN tests at 5, 7, and 10 weeks of age indicated a high degree of immunity to infectious bronchitis.

The other portion of colloid material was prepared as a vaccine in small 20 ml. brown bottles and sent out in the field for replacement pullet flocks. The following is the tabulation of these data:

The three flocks which showed no symptoms were reported as having been vaccinated at 6, 9, and 14 weeks of age, respectively. The flock vaccinated at 9 weeks of age was reported as having had a previous respiratory outbreak at 5 weeks of age which had not been diagnosed. This could have been bronchitis.

Although there could be some discrepancy in these results, since they are field reports made by many different observers, the main objective of this test, which was to determine the infectivity of the colloid material, was established. Osterized material was also used with equally good results.

An experiment was conducted by serially passing the IBV through embryonated duck eggs. Seven serial passages were made but the harvests were of very small amount and the material did not produce immunity to IB. It was assumed that under these conditions the IBV was not adapted to duck eggs.

* Eppenbach Inc., L.I. City, N. Y. ¼ H.P. motor, 8000 rpm.

† Take: Clinical symptoms of coughing and sneezing within 7 days.

TABLE 10

Chicken Egg Embryo Mortality Pattern of a Field Strain of Infectious Bronchitis Virus when Passaged Serially

Egg embryo passage	No. eggs injected	Condition at 48 hours*		Per cent mortality at 48 hours
		Dead	Alive	
7	15	5	8	38.5
8	15	2	7	22.2
9	16	2	11	17.6
10	28	7	14	33.3
11	21	4	13	23.5
12	25	6	15	28.4
13	18	5	9	35.7
14	17	6	9	40.0
15	15	7	4	63.6
16	20	7	8	46.7
17	20	8	6	57.1
18	21	4	3	57.1
19	26	14	5	73.7
20	30	23	2	92.0
21	25	18	3	85.6
22	25	10	5	66.3
23	26	10	9	52.7
24	25	16	6	72.7
25	30	19	6	76.0
26	20	9	4	69.2
27	25	14	9	60.0
28	20	3	17	15.0
29	20	6	14	30.0
30	20	8	12	40.0
31	20	6	14	30.0
32	20	4	15	20.5
33	20	5	15	25.0
34	20	8	12	40.0
35	20	8	12	40.0
36	20	3	15	16.5
37	15	Results not recorded		
38	20	10	10	50.0
39	20	10	9	51.0
40	20	7	13	35.0
41	20	6	13	32.0
42	20	7	12	36.0
43	20	12	8	60.0
44	20	15	5	75.0
45	20	7	13	35.0
46	20	15	5	75.0
47	20	4	13	20.0
48	20	5	15	25.0
49	20	2	14	10.0
50	50	32	13	64.0

* If the number dead and number alive do not add to the total injected, the deficiency is due to those dead and removed at the 24 hour candling.

Flock	No. birds	No. flocks
Symptoms		
Mild	13,124	12
Average	43,710	23
Severe	5,050	4
None	3,400	3
All flocks	65,284	42

Summary and Conclusions

1. Infectious bronchitis in domestic chickens has been widespread in Maine since 1945. The natural incidence of the disease in the state is 85 per cent unless birds are immunized against it.
2. The University of Maine attempted over a 10-year period to provide a vaccination program for Maine poultrymen as well as obtain scientific information about the disease.
3. In field and laboratory studies on 6,209,850 birds from 3395 flocks the severity of the disease, duration of symptoms, and extent of mortality were established.
4. The symptoms were mild 23.5 per cent, average 48.5, severe 15.7 and no symptoms 12.3 per cent of the time.
5. The duration of symptoms were 1 week 21.0 per cent, 1-2 weeks 44.0 per cent, 2-3 weeks 20.8 per cent and over 3 weeks in 14.2 per cent of the flocks.
6. In over six million birds the mortality, including all causes, up to three weeks after vaccination was less than one per cent. This is conclusive evidence that infectious bronchitis vaccination does not produce high mortality.
7. Evidence has been accumulated that although practically all laying birds are immunized as chicks there has been no general drop in egg production or hatchability throughout the state of Maine.
8. Chicks vaccinated in the boxes at one day of age showed 65 per cent to be immune 16 weeks later.
9. Replacement pullet chicks vaccinated at 5 weeks of age or older were still immune one year later.
10. Chicks from immune hens showed a high degree of immunity up to seven days of age, but only 50 per cent were immune by 14 days of age. By 21 days only 10 per cent still maintained parental antibodies against the disease.
11. Under average management conditions 125,000 day-old broiler chicks did not show a higher than normal mortality when vaccinated at one day of age.
12. In broilers vaccinated day-old the serial passage of virus from 7 through 51 seemed to have little effect on severity of disease or immunity produced.

From field and laboratory experience with 721,500 vaccinated chicks it was evident that immunity was established but the success of the program depended on the incidence of secondary bacterial infections, CRD and air sac infections.
13. Laboratory studies did not reveal that the virus became egg adapted

even after 50 serial passages. The whole embryo appeared to be as infective as embryonic fluids alone.

14. The whole study was carried out with the so-called "Maine Strain" of infectious bronchitis virus. Although the original strain came from Massachusetts in 1945, due to zig-zag passages in birds and embryos, it is not known whether the strain is identical today to the Massachusetts strain. The strain has been passed only in chicken egg embryos since 1950.

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BRONCHITIS IMMUNIZATION REPORT SHEET*
ANIMAL PATHOLOGY BUILDING
UNIVERSITY OF MAINE
ORONO, MAINE

PLEASE FILL OUT AND RETURN IN APPROXIMATELY
THREE (3) WEEKS

1. Flock owner.....Address.....
2. Date of inoculation.....Virus lot No.....
3. Source of inoculated stock.....
4. Breed of inoculated stock.....
5. No. of birds in inoc. flock.....Age when inoc.....wks.
6. How severe symptoms: Mild.....Average.....Severe.....
7. How long did symptoms last?.....wks.
8. Location of birds during symptoms: House.....Range.....
9. Did the flock encounter any other disease while having
bronchitis
10. Weather conditions during symptoms: Damp.....Fair.....
11. How many birds died from the effects of bronchitis?.....
(Give per cent if known)
12. Method of inoculation used: Spray.....Swab.....

Return address: BRONCHITIS CONTROL SERVICE
Animal Pathology Building
University of Maine
Orono, Maine

Date of this report.....Signed.....
Cooperator

* This is the report which was returned to the Laboratory for field evaluation of an experimental vaccine.