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COVER: One method of mass immunization by spray.

JANUARY 1955

Avian Mass Immunization for Infectious Bronchitis and Newcastle Disease

By W. R. Dunlop and R. G. Strout*

Introduction

THE PHENOMENAL growth of the poultry industry in the last decade, manifested in size of flocks rather than in number of flocks, presents the need for an entirely new approach to poultry disease control.

Areas of heavy poultry population lend themselves, unfortunately, to rapid spread of acute diseases. An example of this is readily observed in the prevalence of respiratory conditions, such as infectious bronchitis and Newcastle disease. The control of the diseases over the entire country presents a very pressing problem. In spite of the highly specialized techniques of vaccinations carried on today, this problem is increasing in magnitude.

It is apparent that in keeping pace with the industry of today, a method of mass immunization is needed. The arduous task of vaccinating individual birds not only presents an expensive labor problem but also invites a factor known as human error. This factor is considered by some to be largely responsible for the inadequacy of avian immunization programs.

Mass immunization, whether a dust or a spray, is based on one of the soundest principles in the study of the spread of virus diseases. Specifically, it is understood that most virus diseases of the respiratory tract are spread by means of the virus particles riding on dust particles or moisture droplets in the air.

Review of Literature

In 1948, Hitchner and Johnson (7) reported the use of the B_1 strain of Newcastle virus in the intranasal vaccination of day-old chicks. While the duration of immunity was not established, day-old vaccinated chicks were immune at 4 months of age. It was also demonstrated that the vaccination of laying birds did not produce any marked drop in egg production. Attempts to immunize baby chicks by atomization of the B_1 virus resulted in a high morbidity and mortality, but there was no evidence of paralytic symptoms.

Later, Bankowski (1) concluded from laboratory and field vaccination trials with an attenuated Newcastle virus that the length of exposure to the air-borne virus and the age of the chickens being vaccinated are important considerations. The results were of a highly encouraging nature.

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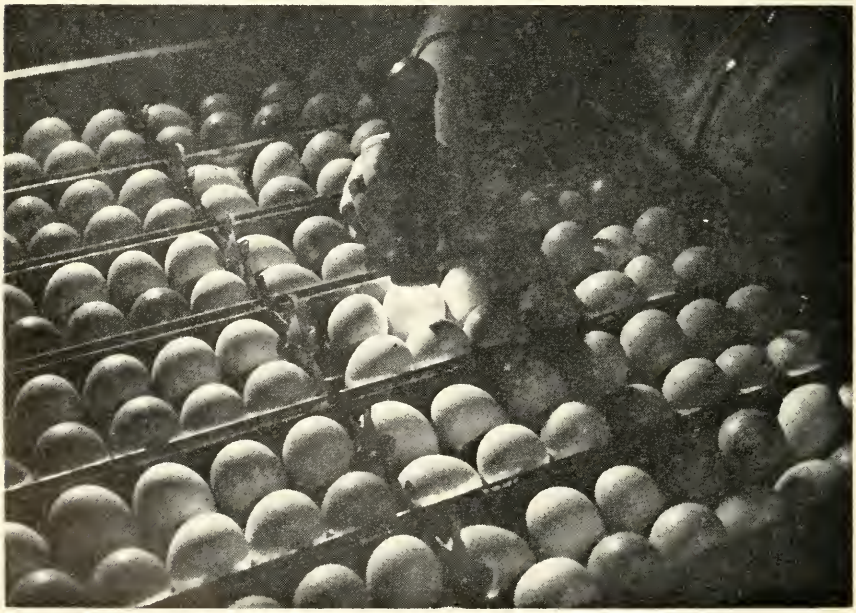
Hitchner in 1950 (5) observed that parental immunity may be overcome in day-old chicks, and that immunity from day-old vaccination may be obtained even in the face of parental immunity.

Hitchner and Reising in 1952 (6), however, demonstrated by laboratory and field trials that atomization of the B_1 strain of virus offered a practical method for vaccinating and revaccinating birds 3 weeks old or older. By such administration, successful revaccination was accomplished in broiler flocks 4 weeks old after intranasal vaccination at one day of age. Replacement flocks revaccinated at 15 to 20 weeks of age demonstrated a good antigenic response.

It was noted also by Hitchner and Reising in this same study that the B_1 strain of virus produced a greater reaction when administered to chicks as a spray than when administered intranasally. The respiratory reaction varied from flock to flock with negligible mortality except in those cases where it appeared that a concurrent infection was present.

Luginbuhl (9) was one of the first workers to combine the vaccinations of Newcastle and infectious bronchitis at an early age. The administration of this vaccine involved the intranasal route applied to each bird.

Crawley in 1953 (2) and 1954 (3) has demonstrated that chicks could be inoculated by a spray technique from one day of age up to 20 weeks. It was observed that the combination of infectious bronchitis virus and Newcastle disease virus into one vaccine produced less interference in growth when the 2 vaccines were applied at the same time than if given separately In regards to Crawley's work it has been found by many investigators difficult to duplicate Canadian results under American field conditions.



Candling embryos in preparation for vaccine production.

Experimental Materials

Newcastle Disease Virus

The strain of Newcastle disease virus used was the B_1 or Blacksburg strain. In the field trials (with one exception, in which a commercial vaccine was used), the avian embryo titre of this virus was $10^{-9.50}$. This strain was obtained directly from Hitchner in 1951.

Infectious Bronchitis Virus

From observations of field outbreaks of infectious bronchitis, it was quite apparent that variations in strains occurred.

In 1951, fifteen strains of infectious bronchitis were isolated, and for the remainder of that year and up to the fall of 1952, repeated screenings of these strains were carried out. This work resulted in the selection of one strain which appeared to be adaptable to mass immunization procedures. This strain was found to have high antigenic properties.

Screenings of embryo passages were carried out and it was found that the twenty-fourth and twenty-fifth embryo passages were desirable for the field trials. The avian embryo titre was $10^{-5.5}$, using one tenth ml. of inoculum per embryo.

Sprayers

Four different sprayers were used in the field trials.

1. Binks power sprayer (electric)
2. Aerosol hand sprayer
3. Common hand fly sprayer
4. Z & W hand sprayer

The type of sprayer first mentioned was used in the application of the commercially dehydrated Newcastle vaccine and, later, in the application of the experimental combination of Newcastle and infectious bronchitis viruses under the direction of a commercial concern.

The three latter types of sprayers were used in conjunction with the experimental vaccine prepared in the University of New Hampshire laboratories.

Diluents

The diluents used in the experiments were modified buffered saline solutions.

Chicks

The chicks used in the experimental laboratory trials were obtained from the poultry department at the University of New Hampshire.

The chicks used in the field trials were provided by cooperating poultrymen in the state of New Hampshire.

Experimental Procedures

Field Trial Procedure

Due to the fact that the University of New Hampshire did not have sufficient facilities for lyophilization of the viruses involved, the vaccines

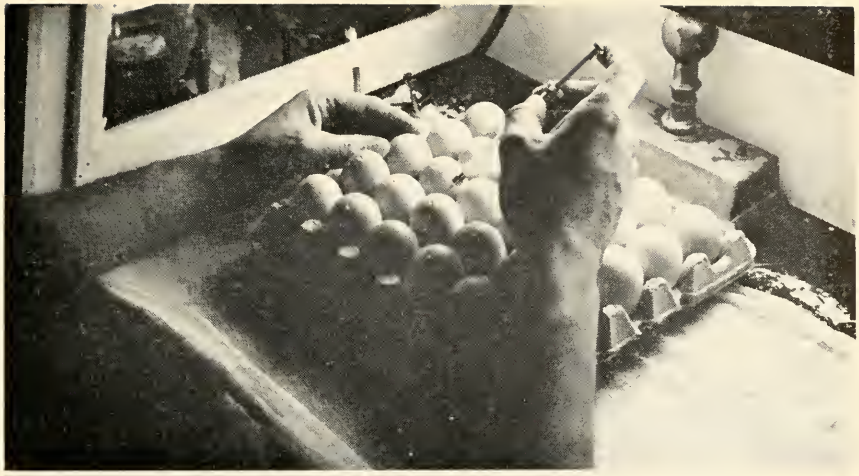
used were maintained in a frozen state until time of use. Commercial dehydration of similar strains of infectious bronchitis did not materially affect the response to the vaccination. In the majority of trials the vaccine was mixed at the farm by a representative of the poultry department. As the volume increased in the state, however, it was necessary in many instances to mix the vaccine beforehand. Such vaccine was picked up at the laboratory by the farmer or field service man. No difference in results was noted between those flocks sprayed by a member of the University or those sprayed by the poultryman himself.

Due to the ease of more complete coverage, the majority of flocks spray-vaccinated were done so at night when the chicks were bedded down. Night administration was later observed not to be necessary providing complete coverage of the chicks could be accomplished. This factor of successful daytime inoculation depended on the type of brooding facilities encountered.

On those farms utilizing space heaters, the fans were turned off during administration of the vaccine. The type of brooding equipment used did not affect the results of the vaccination.

In the case of birds being carried through the growing period to the laying period, it was found necessary, as with commercial vaccines, to revaccinate. This revaccination was carried out at night when the birds were bedded down or had gone to roost. Simply spraying the vaccine over the heads of the birds was all that was necessary to restimulate immunity which

Drilling and disinfecting the embryos prior to inoculation.



Inoculation of the embryos with virus for vaccine production.

lasted through the laying year. Individual handling of the birds was not necessary.

Blood samples were taken from the chicks at the time of vaccination. An attempt was made to procure samples at four-week intervals thereafter. As the volume increased, however, samples were taken from different flocks at different ages by random sampling.

Explanation of Serological Tests

The H. I. test employed in this work has been used as a method of determining antigenic response to vaccination with the B_1 strain of Newcastle virus. This test is of the beta type, described by F. S. Markham. It has been shown by challenge with a known virulent strain of virus that negative tests are not a reliable indication of susceptibility.

The geometric mean titre is a method used in experimental and field studies in serology, and is employed when it is desired to compare one group with another. Unlike the average, which may be entirely misleading, the geometric mean titre has a fixed value.

The serum neutralization test used was a variation of the test described by Fabricant (4). A serum dilution of 1:10 was made and mixed with 10, 100, and 1000 neutralizing doses of embryo-adapted infectious bronchitis virus. Three embryos per dilution were used. Results were considered positive when two out of three embryos in the dilution of 100 or more neutralizing doses lived.

The same test was used in the detection of Newcastle antibodies, except, of course, an embryo-adapted strain of Newcastle virus was used as antigen.

In challenging for infectious bronchitis, a field strain of bronchitis virus was used. This strain underwent a bird titre. It was found that a dilution of 10^{-6} was the end point of infectivity in susceptible birds. A dilution of 10^{-3}, or 1000 infective doses, was applied, .2 cc. intranasally and .2 cc. intratracheally.

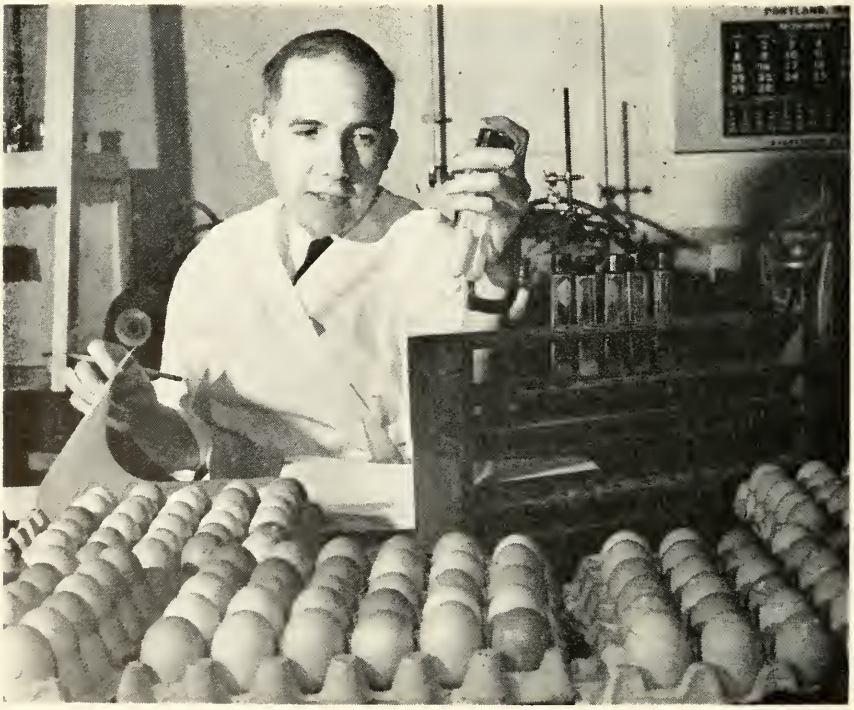
The challenge for Newcastle immunity was carried out by injecting .5 cc. of 10 mld's (obtained by bird titre) of the Boney strain of virus intramuscularly. Birds showing symptoms of paralysis were considered as dead.

Preliminary Laboratory Results

Thirty chicks, parentally immune to Newcastle disease, were used in the first laboratory trial. The chicks were sprayed on the second day. A respiratory "take" occurred 5 days later which lasted for an additional 5 days. At 5 weeks, blood samples were taken for an H. I. and S. N. test, and the entire group was challenged with 10 mld's of the Boney challenge strain of Newcastle virus. Two birds died and one showed paralysis. Seventy and one-half (70.5) per cent of these birds showed positive H. I. titres while 79.3 per cent showed positive neutralization titres.

During this same period laboratory trials were being run on the selected strains of infectious bronchitis mentioned previously. At this juncture it was decided to combine Newcastle and infectious bronchitis vaccines. The Blacksburg strain of Newcastle virus and selected strains of infectious bronchitis were used in these trials.

Four parallel groups of 30 susceptible 4-week-old chicks were sprayed with combination Newcastle and infectious bronchitis strains. In addition, 2 groups of 30 chicks, 3 and 4 days of age, parentally immune to Newcastle and infectious bronchitis were sprayed.



Recording sterility and potency.

The respiratory take was apparent in the younger chicks on the third or fourth day, being quite mild until the fifth day. The 4-week-old birds showed a take on the second day. The length of respiratory take in all groups was about 15 days. No mortality appeared in the older birds during this time while one chick was lost in the younger group.

All birds were challenged 4 weeks later. One half were challenged by the intratracheal route with 1000 infective chick doses of a field strain of infectious bronchitis virus. The second half were challenged with the Boney challenge strain of Newcastle virus as previously described. Two birds died as a result of the Newcastle challenge and one showed paralysis. Three birds showed mild respiratory symptoms for two days when challenged with infectious bronchitis.

Following this trial a further laboratory test was conducted on some 600 chick divided into 6 pens. The parent stock was vaccinated with a field strain of infectious bronchitis virus and with wing web vaccine for Newcastle disease just as they came into production. Random blood samples were taken from all groups of chicks and it was apparent that parental immunity existed for Newcastle disease and infectious bronchitis.

The birds were vaccinated with combination Newcastle and infectious bronchitis vaccine by the spray technique. The take was mild on the second and third days. About the fifth day the take was quite apparent. Respiratory take had disappeared from all groups by the 17th day.

At 8 weeks random blood samples were drawn for serological test. On Hemagglutination Inhibition test 63.3 per cent showed positive H. I. titres, while on serum neutralization test for infectious bronchitis 76.8 per cent showed positive titres.

At 16 weeks only 55.1 per cent showed positive H. I. titres for Newcastle disease; 68.8 per cent were capable of neutralizing better than 100 embryo lethal doses of infectious bronchitis virus.

Experimental Results

Spraying with UNH Newcastle Vaccine

During a three-month period, 25 flocks involving 67,280 birds were inoculated in the field at 2 to 3 days of age with the B_1 strain of Newcastle virus. The spray technique, utilizing the Z & W type sprayer, as well as others, was used in the administration of this vaccine. The size of the flocks ranged from 550 to 10,000 chicks. Brooding systems of all types were encountered.

In all cases, respiratory symptoms were observed around the fifth or sixth day post inoculation. The average length of response was 6.5 days. The mortality from all causes averaged 1.8 per cent for the first two weeks following vaccination. Blood samples, taken at random, were withdrawn at 4, 8, 12, and 16 weeks, respectively. Geometric mean titres are shown in Table 1. (In the compilation of geometric mean titres for this bulletin, serum samples in the H. I. test were diluted no higher than 1:128. This explains the relatively low figure obtained. It must be remembered here that the geometric mean titre is merely a value obtained for the use of comparison purposes.)

Figure I

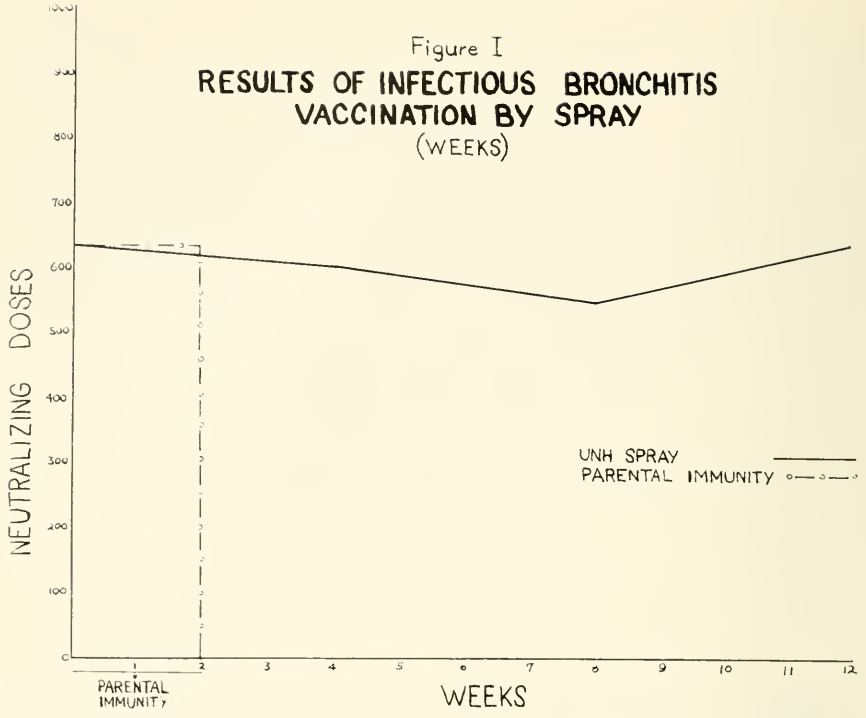


Figure II

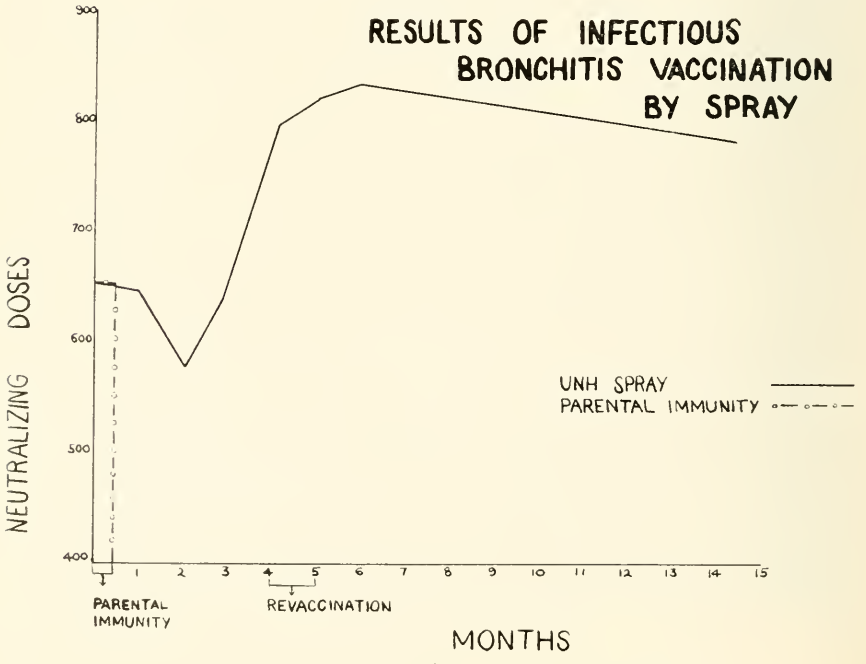


Table 1. Results of UNH Newcastle Spray Vaccine

Age	4 weeks	5-8 weeks	9-12 weeks	13-16 weeks
Geometric mean titre	7.75	6.94	7.22	5.582

Serum neutralization tests indicated that 79.8 per cent of the samples neutralized 100 or more neutralizing doses.

Random samples of birds, withdrawn at the same time for challenge, exhibited a resistance of 87.5 per cent, with 98.8 per cent of the susceptible controls dying.

Spraying of Commercial Newcastle Vaccine

During a period covering approximately 3 months, experiments were undertaken using the Binks power paint sprayer in the administration of a commercially dehydrated Newcastle vaccine.

Approximately 45,750 birds were sprayed at 2 to 3 days of age with this equipment. The mortality from all causes for 2 weeks following vaccination was 1.7 per cent. The duration of the respiratory symptoms was an average of 9 days.

Blood samples, taken at random, were withdrawn at 4, 8, and 12 weeks, respectively. The geometric mean titres are recorded below.

Table 2. Results of Spraying a Commercial Newcastle Vaccine

Age	4 weeks	8 weeks	12 weeks
Geometric mean titre	5.821	5.743	5.133

Unfortunately, due to the lack of adequate space for challenge, this process was omitted.

Serum neutralization tests showed that 80.9 per cent of the samples tested neutralized 100 or more neutralizing doses.

Spraying of Combination Newcastle-Infectious Bronchitis Vaccine (UNH)

Field trials were carried out utilizing the B_1 strain of Newcastle virus in combination with the strain of infectious bronchitis previously described.

The first spray vaccination utilizing the combination of Newcastle and bronchitis vaccines took place on a farm where 100,000 broilers are raised every 12 weeks, and 50,000 breeders are kept each year. Newcastle disease was a very serious problem on this farm, for it had experienced outbreaks of 31 per cent and 85 per cent losses in the past. Infectious bronchitis and chronic respiratory diseases were also a definite problem.

Blood samples, taken at random on 2- or 3-day-old chicks, showed a significant H. I. titre in 88.7 per cent of the samples.

The inoculations took place on either the second or third day of age. Respiratory symptoms were observed on the second day post inoculation and lasted approximately 16.5 days.

Random samples taken at intervals of 4 weeks presented the following geometric mean titres.

Table 3. Results of Newcastle Spray at Farm No. 1

Age	4 weeks	8 weeks	12 weeks
Geometric mean titre	7.727	7.464	7.127

At ten weeks of age, two groups of ten birds were withdrawn for challenge purposes. One group was challenged with 1000 infective doses of a field strain of infectious bronchitis, employing .2 cc. intratracheally. One bird showed respiratory symptoms against challenge. In the other group, which was challenged with the Boney strain of Newcastle virus, two birds showed paralysis.

The mortality from all causes on the 100,000 broilers was 2.3 per cent for three weeks following vaccination.

According to serological test, adequate parental immunity was present in chicks hatched from the spray-vaccinated parents. These chicks were spray-vaccinated, showing a geometric mean titre of 16.14 at day-old, with 95.0 per cent of the samples tested for bronchitis antibodies, neutralizing 100 or more neutralizing doses of an embryo-adapted bronchitis virus.

During the past two years over 3,000,000 birds have been vaccinated in the State of New Hampshire, with over 200 individual farms having been involved. The size of the flocks ranged from 100 to 100,000 chicks. Brooding systems of various types were used on these farms. Management conditions of all types were also encountered. The age of the chicks ranged from 1 to 7 days at the time of vaccination.

The mortality figures on the entire number of birds sprayed averaged 2.4 per cent. This figure includes losses from all causes, not merely losses resulting from the vaccination alone. At one period during the fall and early winter of 1953, the average mortality was 3.1 per cent, but a change in the technique at that time lowered the mortality figures. The figures for the three months following presented an average mortality from all causes of 1.9 per cent.

The symptoms appeared on an average of three days post inoculation. Such symptoms were generally mild and, in isolated instances, went unnoticed. The average length of symptoms was approximately 17 days.

Blood samples, taken at the time of vaccination, and at 4-week intervals thereafter, exhibited the following geometric mean pattern. (Samples labeled as day-old may actually be from 2- to 3-day-old chicks, but in all instances they are pre-inoculation titres.)

Table 4. Results of Newcastle Spray When in Combination with Infectious Bronchitis

Age	(Pre-Inoc.) Day-Old	Weeks					
		4	5-8	9-12	13-16	16-19	20+
Geometric mean titre	15.32	7.52	6.97	8.82	7.22	4.34	18.24
Positives	86.8%	76.7%	72.5%	73.0%	75.6%	70.5%	91.8%

Serum neutralization tests for Newcastle disease antibody, while of necessity very limited, demonstrated that 76.8 per cent neutralized 100 or more neutralizing doses of an embryo adapted Newcastle virus strain at 16 weeks of age.

Figure III

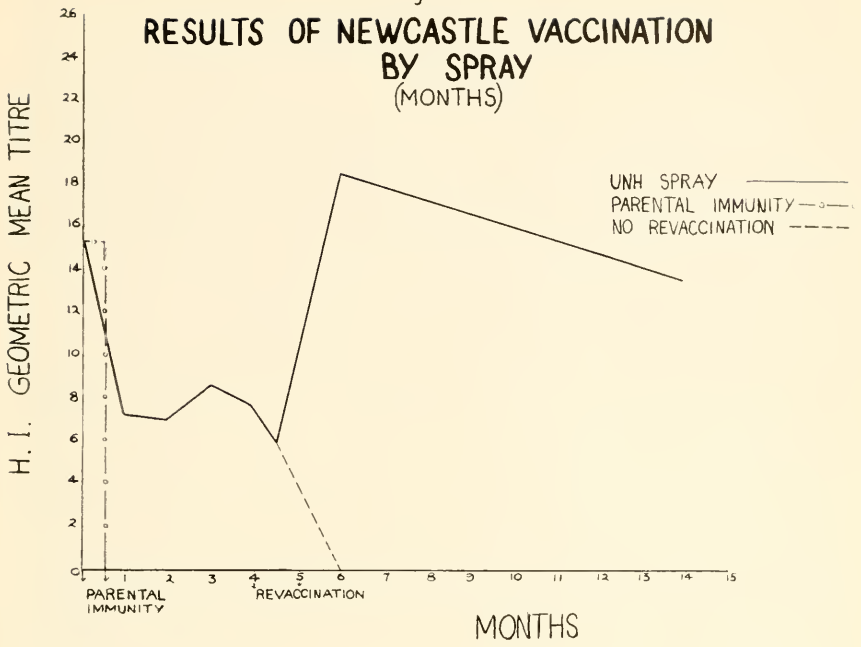
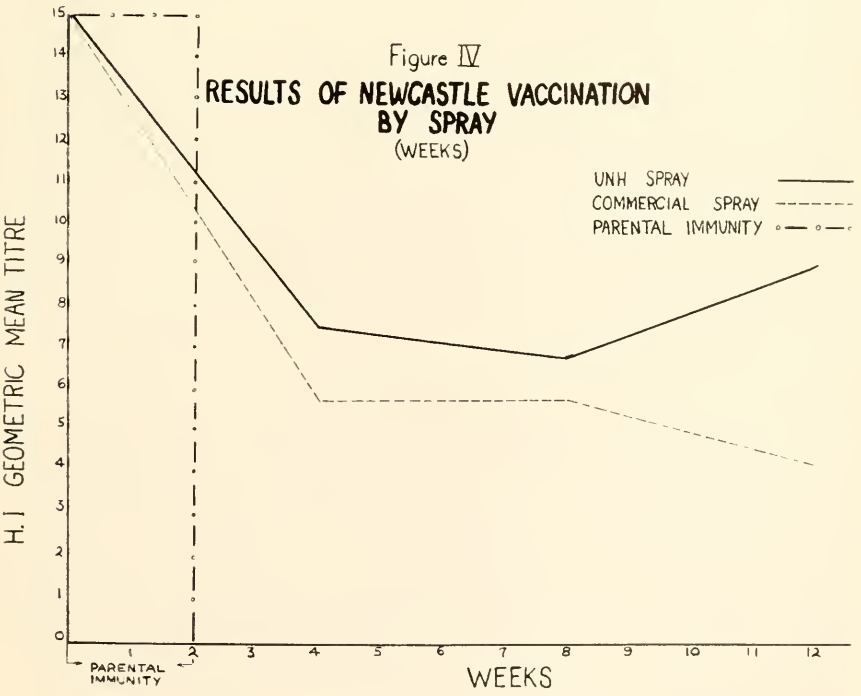


Figure IV



The serology on the infectious bronchitis presented the data indicated in Figures 3 and 4. Individual blood samples were used.

The results on the graphs show that 74.2 per cent of the samples neutralized 100 neutralizing doses or better at day-old: 85.7 per cent at 4 weeks, 84.2 per cent at 5-8 weeks, 89.0 per cent at 9-12 weeks, 73.5 per cent at 13-16 weeks, 96.6 per cent at 17-20 weeks, and 96.7 per cent at 20 weeks or more. The reason for the higher percentage of positives at 16 weeks and beyond is the result of revaccinations carried out from 16 to 20 weeks.

At the time blood samples were withdrawn for serological purposes, birds were also withdrawn for challenge. The ages of the challenge birds ranged from 4 to 16 weeks. The results are shown in Table 5.

Table 5. Results of Bronchitis Challenge

	Vaccinates		Controls	
	Number Birds	Symptoms	Number Birds	Symptoms
Group I	25	0	2	2
Group II	20	1	2	2
Group III	25	0	2	2

Table 6. Results of Newcastle Challenge

	Vaccinates		Controls	
	Number Birds	Died	Number Birds	Died
Group I	25	2	2	2
Group II	20	2	2	2
Group III	25	3	2	2

One hundred thirty-eight chicks, found to be susceptible to Newcastle disease at 4 weeks of age by random sampling, were spray-vaccinated at the age of 4 weeks. Two birds died during the period of symptoms. Twelve random samples taken at 6 weeks of age exhibited a geometric mean titre of 14.25, confirming previous work (8) that older birds develop higher levels of antibody than young chicks. Six samples run on S. N. tests for infectious bronchitis antibody showed that one sample neutralized 10 neutralizing doses, one sample 100 neutralizing doses, and 4 samples 1000 neutralizing doses. The numbers, while of necessity small, indicate susceptible birds produce a higher titre upon vaccination.

Discussion

There is one period in the life of a chicken when carefully controlled factors result in optimum conditions for life and growth. This period, when variables are more constant than at any other time, is when the chicks are being brooded under the hover. It is at this time, when the optimum conditions exist, for any early age vaccinations to be carried out.

With this factor in mind, early field trials of spray vaccination of Newcastle vaccine, or Newcastle in combination with infectious bronchitis vaccine, were carried out from 2 to 3 days of age in some cases. Recent work indicates that any age prior to production may be satisfactory for vaccination, however.

The data presented in this bulletin was gathered entirely from the State of New Hampshire, a state on the eastern seaboard, in which considerable poultry population exists at the present time.

Birds raised on some farms, according to random samples, possess immunity to infectious bronchitis at all times. This situation exists because of a constant exposure to the virus. The majority of breeding farms do not find this situation to exist, and as a result, a field strain of bronchitis is given to the birds prior to coming into egg production. The general picture of breeding flocks in New Hampshire is that practically all flocks have been exposed to bronchitis either by artificial means or by naturally occurring field strains. Consequently, with very few exceptions, chicks hatched from these breeding flocks possess a high parental immunity during the first week of age. Furthermore, it must be kept in mind that such immunity has been stimulated by an unmodified strain. Luginbuhl demonstrated immunity to infectious bronchitis at 5 weeks when the chicks were vaccinated at day-old intranasally with modified infectious bronchitis.

As may be seen from the table, the majority of chicks hatched from New Hampshire breeders possess high parental immunity to Newcastle disease. The practice of applying Newcastle vaccine by the wing web method has been a favorite of the breeders of this state. In the presence of this high parental immunity, however, it has been shown to be possible to infect chicks with the B_1 strain. Hitchner (5) showed that it was possible to do so at an early age, and Markham and Bottorff (10) proved that it was possible to immunize chicks by use of the intranasal or intraocular route in the presence of parental immunity.

Another important factor to be mentioned in connection with the previous results is that of chronic respiratory disease. It has been determined by the observation and study of pipped embryos which showed enlarged hocks and caseous material in the air sacs, also by isolation of CRD agents from day-old chicks, that the incidence of CRD in the chicks hatched in New Hampshire is exceedingly high. In those instances in which high mortality was encountered in the field trials, CRD was found to be present in over 90 per cent of the cases.

While it was originally felt that the chicks to be spray vaccinated must possess parental immunity to infectious bronchitis, results of field trials and laboratory trials on susceptible chicks would seem to indicate that parental immunity, while desirable, is not necessary. From all observations, the indications are that the CRD complex presents the biggest problem in affecting the results of any mass immunization or other vaccination procedures today.

Summary

1. It has been demonstrated that mass immunization of young chicks with the B_1 strain of Newcastle virus, employing spray techniques, is a safe method, and will produce sufficient immunity to withstand 10 minimum lethal doses of Boney Newcastle disease challenge virus at approximately 12 to 16 weeks of age.

2. It has been demonstrated that there is no apparent interference involved in the combination of Blacksburg strain of Newcastle and the UNH strain of infectious bronchitis viruses when such a combination vaccine is administered to young chicks utilizing the spray method of mass immunization.

3. It has been demonstrated that mass immunization by the spray methods of a combined Newcastle-bronchitis vaccine is a safe method of vaccination and will produce sufficient immunity to withstand 10 minimum lethal doses of Boney Newcastle challenge and 1000 infective doses of field strain of infectious bronchitis up to approximately 12 to 16 weeks of age.

4. It has been demonstrated that the spray method of mass immunization is an easy and rapid method of vaccination. One man may vaccinate up to 10,000 chicks per hour, using this method, depending on brooding conditions.

References

1. Bankowski, R. A. 1950. Further Studies *in vitro* Cultivated Pneumoencephalitis (Newcastle Disease) Virus and Its Use as a Vaccine, Vet. Med. 45: 322-327.

2. Crawley, J. F. 1953. Spray Immunization of Broiler Chicks with Combined Bronchitis—Newcastle Disease Live Virus Vaccines. 25th Annual Conf. Lab. Workers Pullorum Disease Control.

3. Crawley, J. F. and Fahey, J. E. 1954. The Spray Method for Bronchitis and Newcastle Disease Vaccination. Southwest Vet. 7.

4. Fabricant, J. 1951. Studies on the Diagnosis of Newcastle Disease and Infectious Bronchitis of Fowls. IV. The Use of the S N Test in Diagnosis of Infectious Bronchitis. Cornell Vet. 41: 68-80.

5. Hitchner, S. B. 1950. Further Observations on a Virus of Low Virulence for Immunizing Fowls Against Newcastle Disease (Avian Pneumoencephalitis). Cornell Vet. 40: 60-70.

6. Hitchner, S. B. and Reising, G. 1952. Flock Vaccination for Newcastle Disease by Atomization of the B_1 Strain of Virus. Proc. Bk. Am. Vet. Med. Assoc.: 258-264.

7. Hitchner, S. B., and Johnson, E. P. 1948. A Virus of Low Virulence for Immunizing Fowls Against Newcastle Disease (Avian Pneumoencephalitis). Vet. Med. 43: 525-530.

8. Jungherr, E. L. and Terrell, N. L. 1948. Naturally Acquired Passive Immunity to Infectious Bronchitis in Chicks. Am. Jour. Vet. Res. 9:201-205.

9. Luginbuhl, R. E. 1952. Studies on Serologic Diagnosis and Immunization of Avian Infectious Bronchitis. Master's Thesis, University of Connecticut, Storrs, Connecticut.

10. Markham, F. S., Bottorff, C. A., and Cox, H. R. 1951. The Conjunctival Application of Newcastle Disease Vaccine (Intranasal Type) in Parentally Immune and Susceptible Chicks. Cornell Vet. 41: 267-282.