

2-1-1964

TB9: Newcastle Disease Virus Activity and Volume of Amniotic Allantoic Fluid in Chicken Embryos from Flocks with Different Vaccination Histories

Harold L. Chute

D. C. O'Meara

D. B. Reardon

H. T. Wheelwright

Follow this and additional works at: https://digitalcommons.library.umaine.edu/aes_techbulletin



Part of the [Poultry or Avian Science Commons](#)

Recommended Citation

Chute, H.L., D.C. O'Meara, D.B. Reardon, and H.T. Wheelwright. 1964. Newcastle disease virus activity and volume of amniotic allantoic fluid in chicken embryos from flocks with different vaccination histories. Maine Agricultural Experiment Station Technical Bulletin 9.

This Article is brought to you for free and open access by DigitalCommons@UMaine. It has been accepted for inclusion in Technical Bulletins by an authorized administrator of DigitalCommons@UMaine. For more information, please contact um.library.technical.services@maine.edu.

UNIVERSITY OF MAINE

THE MAINE AGRICULTURAL EXPERIMENT STATION

ORONO, MAINE

**Newcastle Disease Virus Activity
and Volume of Amniotic Allantoic Fluid
In Chicken Embryos
From Flocks with Different Vaccination Histories**

H. L. Chute
D. C. O'Meara
D. B. Reardon
H. T. Wheelwright

GOVERNMENT DOCUMENTS AND MICROFORMS DEPARTMENT
UNIVERSITY OF MAINE
5729 FOGLER LIBRARY
ORONO, ME 04469-5729

Bulletin T 9
Technical Series

February
1964

NEWCASTLE DISEASE VIRUS ACTIVITY
AND VOLUME OF AMNIOTIC ALLANTOIC FLUID
IN CHICKEN EMBRYOS
FROM FLOCKS WITH DIFFERENT VACCINATION HISTORIES

by

H. L. Chute, D.V.M., M.S., D.V.Sc., D. C. O'Meara, A.B., M.S.,
D. B. Reardon, and H. T. Wheelwright

Summary

Breeding hens of similar age, 9 to 11 months old, were selected from four flocks, each with a different vaccination history. One flock received no vaccinations, the second received spray and wing web Newcastle disease (ND) vaccine, the third was given three ND dust vaccinations, and the fourth received two dead ND viral vaccinations.

From the Department of Animal Pathology, University of Maine, Orono, Maine

A portion of this report was presented at the 100th Annual Meeting of the American Veterinary Medical Association, New York, N. Y., July 28 - August 1, 1963.

Supported in part by Northeast Regional Research funds under project number NE 5.

Thirty dozen eggs were randomly selected from each flock on the same day and incubated. At 10 days incubation half of the eggs were injected allantoically with B₁ (lentogenic) strain and the remaining eggs with Texas-GB (velogenic) ND virus strain. Forty-eight hours later the total allantoic (aa) fluids were harvested and measured. Hemagglutination (HA) activity and the embryo lethal dose 50 per cent mortality (ELD₅₀) were determined on all dilutions of both viral strains.

Blood samples were taken from the three vaccinated flocks at the time the eggs were collected and hemagglutination-inhibition (HI) titers determined.

Considerable variation in HA activity existed among fluids of inoculated eggs from the same flock. The ELD₅₀ titer was two logarithms higher in the eggs from the non-vaccinated flock using the Texas-GB strain of virus. The embryo infective dose (EID) as measured by HA was greatest in the non-vaccinated birds and as much as four logarithms lower in one vaccinated group. The volume of allantoic and amniotic fluid per embryo varied from 2 to 18 ml. with average yields on a flock basis from 7 to 13 ml. per egg.

Introduction

Chicken embryos in biological studies have been used extensively for the past 60 years. However, after 1933 (Goodpasture)

its use in microbiology has been paramount. Since 1946 (Burnet and Beveridge) virology has used the chicken egg embryo more than any other scientific discipline. Our own laboratory has used approximately 50,000 fertile chicken embryos annually for the past 13 years.

During this time little study has been given the differences in quality of the eggs. Commercial poultrymen are very familiar with the fact that there is a great difference in fertility, hatchability, and marketability of chicks from different egg sources.

At present the chicken egg embryo is used for the propagation and titration of animal and human viruses, pharmaceutical testing, vaccine and bacterin production, isolation and identification of new viruses and other uses too numerous to mention.

Unfortunately, too little attention has been given to the source and quality of these eggs. It is a well known fact that the fertility varies between different strains of birds as well as the hatchability. Besides this, eggs are notorious for the transovarian transmission of numerous bacteria and viruses.

The purpose of this experiment was to ascertain if any differences existed in the propagation of two strains of Newcastle disease virus (NDV) in eggs from birds with different vaccination histories.

Although the utilization of chicken egg embryos for virological studies has been used for about 30 years it is only during

the last 15 years that extensive studies and large numbers have been used. During this latter time the pharmaceutical industry has used large numbers for the production of many veterinary and human biological products. The chicken egg embryo offers an economical and convenient method for the pursuit of many fundamental viral investigations such as optimal conditions for the cultivation, effect of physical, chemical and biologic agents, serological tests, immunogenic properties and pathological effects.

Review of Literature

Nadel et al (8) by using the Poisson distribution based on assumed minimal numbers of viral particles determined the number needed to initiate infection. The statistical determinations were compared to actual values derived from simultaneous inoculations of embryonated eggs and 2-day-old chicks with various dilutions of virus. They found that one virus particle in the inoculum or a successful attack of one locus was sufficient to kill a 10-day embryonated egg, but from two to four, or possibly five, Newcastle disease viral particles were needed to kill the 2-day-old chick.

Upton et al (11) studied the antigenic differences among strains of Newcastle disease virus (NDV). They found that from 17 heterologous combinations of NDV and antiserum in serum neutral-

ization tests that B₁ antiserum exhibited a difference of greater than 1.0 log in its capacity to neutralize the homologous and the heterologous viral strains.

Monti (7) studied the hemagglutination-inhibition and the viral neutralization antibodies in the egg yolk of fowls vaccinated and hyperimmunized against ND. He found that, when compared with that of the blood, the titer of ND virus neutralization antibodies was very low in the yolk of eggs from fowls which had been vaccinated with formalized fowl embryo vaccine or with live attenuated virus. The hemagglutinating antibodies were present in almost equal concentrations in the blood and egg yolk.

Gagliardi in 1960 (5) concluded that NDV grown in eggs from non-immunized hens does not undergo biologic changes when mixed and homogenized with the corresponding egg yolks; if grown in eggs from hens with specific immunity it lost some of its infectivity and immunogenicity when mixed and homogenized with the corresponding yolks and this is in relation to the varying degrees of specific antibodies present in the yolks.

Rauscher et al (9) studied the effect of 17 viruses inoculated via different routes into Japanese quail (Coturnix coturnix japonica) embryos and compared reactions to those found in chicken egg embryos. These workers failed to mention the source or breed of the chicken eggs. Apparently, they used only one source of chicken eggs. They found little difference in the growth

characteristics of the viruses studied in the Japanese quail eggs and those in chicken eggs.

Keeble and Wade (6) studied an inactivated ND vaccine in England and found a close relationship which was linear between the geometric mean serum-neutralizing antibody titers for all birds in a group having the same number of HI units per ml.

Materials and Methods

Standard methods of virology as described by Cunningham (4) were used. The chicken embryos were incubated 10 days at 37°C prior to use. HA and HI techniques were those of the alpha method (13).

The chicken eggs were obtained from four different sources and the hens were as close to the same age as possible. Thirty dozen eggs from each flock arrived at the laboratory so that they were all set in the incubators the same day, December 5th, 1963. They were clean unwashed eggs. Ten hens from each of the flocks except "A" were bled aseptically from the heart the day the eggs were obtained. The history of the flocks were as follows:

Farm A. These were SPAFAS Inc. eggs from Norwich, Conn. This White Leghorn flock had never been vaccinated for any of the infectious diseases and was negative to tests for the following: infectious bronchitis, Newcastle disease, avian encephalomyelitis,

laryngotracheitis, fowl pox, and Celo virus. This was essentially a negative control source of eggs.

Farm B. This flock consisted of Vantress males and Arbor Acres line 50 females. They were hatched February 26, 1962. The Newcastle vaccination was a spray B₁ vaccine at 14 days of age and wing web vaccinated with MK107 strain of vaccine at 14 weeks of age. They were sprayed with a DG type infectious bronchitis (IB) vaccine at 3 and 15 weeks of age respectively. The flock received a fowl cholera bacterin at 14 weeks of age.

Farm C. This flock was of the same breeding as Farm "B". They were hatched February 26, 1962. This flock received dust vaccine at 1, 5 and 26 weeks of age respectively. The vaccine contained a B₁, NDV and a DG type IBV. At 29 weeks of age they received an intra-ocular experimental infectious laryngotracheitis (ILT) vaccine.

Farm D. This flock was also of the same breeding as Farm "B". They were hatched April 5, 1962. The flock was vaccinated with inactivated NDV (Maine Biological Laboratories, Inc., Waterville, Maine) at 15 and 20 weeks of age respectively. They were IB vaccinated with a spray DG type vaccine at 6 and 20 weeks of age respectively. The only other vaccination was a fowl cholera bacterin at 20 weeks of age.

Virus Strains. Two strains of NDV were used. (1) GB Texas strain - (2) B₁ strain.

Each source of eggs was marked, incubated 10 days, and removed for inoculation with the ND virus. 0.2 ml. of the respective dilutions of the ND virus 10^{-1} through 10^{-14} were inoculated into the allantoic cavity. A minimum of 5 eggs per dilution were used. At 24 hours the embryos were candled and the dead ones removed and discarded. At the end of 48 hours each egg was individually harvested by pouring off the allantoic and amniotic fluids by rupturing the chorioallantoic membrane into a screened funnel and a pool was made from each dilution. For the GB Texas strain the method of Reed and Muench (10) was used to determine the LD_{50} end-points. For the B₁ strain an EID was determined by performing the hemagglutination test and the highest dilution at which activity was found was determined to be the end-point.

Usually titrations were run 10^{-1} through 10^{-14} . At 5 eggs per dilution this would only use 70 of the possible 360 eggs which were incubated. However, not all of the eggs were fertile and only healthy appearing embryos were used for injection. The excess served as uninjected controls.

Experimental Results

Table I shows that flock "A" was serologically negative to all the common virus diseases. (These results obtained from Dr. R. Luginbuhl, University of Connecticut, Storrs, Connecticut).

Flocks B, C, and D all showed serological titers to ND.

Table II presents the LD₅₀ test results with the Texas-GB strain of NDV. The evidence is clear that a higher LD₅₀ is obtained from eggs from non-vaccinated hens than from any of three different vaccinated groups.

Table III records the BID as determined by HA activity. Here again the highest activity was with fluids from eggs of non-vaccinated sources. These data indicate that the residual congenital antibody of the egg yolk inhibits the growth of the virus in chicken embryos.

The charts in the appendix show the individual egg HA titers of the aa fluid as well as the pooled HA activity of each virus dilution.

Table IV shows that the average volume of aa fluid from inoculated embryos varied from 7 to 11 ml. The higher the pooled HA activity the less the amount of fluid.

Discussion

There is considerable evidence that the egg not only transmits numerous pathogenic agents, but also transmits parental antibodies. Brandly et al (1) found that specific antiviral or antibody activity was higher in yolks of eggs laid by ND vaccinated hens. Van Roekel et al (12) studied the epizootiology of PPLO in-

fection and concluded that, among the modes of transmission, transovarian infection was the most common method of perpetuating the disease from one generation to the next.

As more critical analyses are made relative to the growth and activity of viruses in chicken embryos, researchers must be more careful in the selection of eggs for such studies. In fact, the source of embryos must be carefully selected and studied. Besides serological and laboratory studies it will be desirable to make frequent clinical examinations of the flock.

Before studying any avian virus in avian embryos, the researcher should ascertain whether the flock has ever had this virus disease and preferably seek eggs from uninfected sources. Since it is possible to produce birds relatively free of the common poultry diseases, (2) (3) it seems reasonable that all laboratories should strive to use only chicken embryos from maximally specific disease-free hatching egg flock sources for use in any testing procedure.

Research from this laboratory indicates that chicken egg embryos should be negative to antibodies for the disease which is being studied. This means that the parent hen should not be vaccinated and should have no antibodies for the particular disease in question. This is particularly applicable to the virus diseases such as ND, infectious bronchitis or infectious laryngotracheitis. The source of hatching eggs is very important for any

valid study using chicken egg embryos.

Conclusions

1. The ELD_{50} of a Texas-GB (velogenic) strain of NDV was highest in the fluids of embryos from non-vaccinated birds. In three different types of vaccination it was at least one log. higher in two groups and two logs. higher in another group of eggs.

2. The embryo infective dose as measured by hemagglutination activity (HA) was highest in embryos from non-vaccinated birds. It was four logs. higher in eggs from non-vaccinated birds than one group of vaccinated birds.

3. Vaccination for Newcastle disease interferes with the growth of NDV in chicken embryos from the vaccinated birds.

4. The volume of aa fluid was less in the higher HA embryos and more in the lower HA activity eggs. The average range was 7 to 11 ml. per embryo but the individual egg variation was from 2 to 18 ml.

TABLE I
HISTORY AND SEROLOGY OF EGG SUPPLY FLOCKS⁽¹⁾

Breed	Hatch Date	Newcastle		Infectious Bronchitis	PPLO	
		Vaccination	Serology	Vaccination	Serology	Other
A. flock White Leghorn	?	None	Negative	None	Negative	AE and CELO Negative
B. flock VT X AA50	2/26/62	#1 = spray 2 wks. #2 = wing web 14 wks.	12/3/62 10 SN Pos.(3) 10 HI Pos.(4)	Spray #1 = 3 wks. #2 = 15 wks.	10 Pos.	Fowl Cholera bacterin 14 wks.
C. flock VT X AA50	2/26/62	Dust #1 = 1 wk. #2 = 5 wks. #3 = 26 wks.	12/3/62 9 SN Pos. 1 SN Neg. 9 HI Pos. 1 HI Neg.	Dust #1 = 1 wk. #2 = 5 wks. #3 = 26 wks.	10 Pos.	Intra- ocular ILT vaccine 29 wks.
D. flock VT X AA50	4/5/62	Killed ND #1 = 15 wks. #2 = 20 wks.	12/3/62 7 SN Pos. 3 SN Neg. 5 HI Pos. 5 HI Neg.	Spray #1 = 6 wks. #2 = 20 wks.	10 Pos.	Fowl cholera bacterin 20 wks.

(1) Flocks B, C and D were approximately 40 weeks of age when blood samples were taken and the eggs were collected.

(2) VT = Vantress Males. AA50 = Arbor Acres line 50 females.

(3) SN = Serum neutralization test.

TABLE II
 LD₅₀ OF TEXAS GB-NDV FROM
 DIFFERENT HATCHING EGG SOURCES

Farm Source	Vaccination Treatment	LD ₅₀ Virus Titer
A	None	10 ^{-10.83} (1)
B	Spray 2 wks. Wing Web 14 wks.	10 ^{-9.22}
C	Dust 1, 5 and 26 wks.	10 ^{-9.34}
D	Killed ND Vaccine 15 and 20 wks.	10 ^{-8.81}

(1) Log. virus titer

TABLE III
 EMBRYO INFECTIVE DOSE⁽¹⁾ OF B₁ NDV
 FROM DIFFERENT HATCHING EGG SOURCES

Farm	Vaccination Treatment	Highest dilution with HA Activity	
A	None	10 ⁻¹⁰	HA 640
B	Spray 2 wks. Wing Web 14 wks.	10 ⁻⁶	HA 320
C	Dust 1, 5 and 26 wks.	10 ⁻⁹	HA 640
D	Killed ND Vaccine 15 and 20 wks.	10 ⁻¹⁰	HA 160

(1) Hemagglutination activity of pooled allantoic fluid from 5 embryos from each dilution.

TABLE IV
VOLUME OF AMNIOTIC-ALLANTOIC FLUID IN MILLILITERS FROM HATCHING EGGS

Farm	Test Virus	LD ₅₀	Quantity of AA fluid in ml. from different dilutions of test virus													TOTALS			
			10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁹	10 ⁻¹⁰	10 ⁻¹¹	10 ⁻¹²	10 ⁻¹³	10 ⁻¹⁴	No. Eggs	AA Fluid	Average Per Egg
A	GB Texas	10 ^{-10.8}	-	-	-	-	30	34	32	35	35	30(4)	42	28(4)	-	-	38	266	7 ml.
A	B ₁	10 ^{-2.3}	44	44	41	47	33	46	58	46	45	47	-	-	-	-	50	451	9 ml.
B	GB Texas	10 ^{-9.22}	-	-	-	-	40	51	36	47	43	54	-	-	-	-	30	271	9 ml.
B	B ₁	10 ^{-1.56}	62	57	51	55	61	47	58	54	49	61	-	-	-	-	50	555	11 ml.
C	GB Texas	10 ^{-9.34}	-	-	-	-	50	54	56	49	53	43	-	-	-	-	30	305	10 ml.
C	B ₁	10 ^{-1.0}	69	74	59	67	70	58	61	59	60	-	-	-	-	-	45	577	13 ml.
D	GB Texas	10 ^{-8.81}	-	-	-	-	31	53	53	58	65	-	-	-	-	-	25	260	10 ml.
D	B ₁	10 ^{-1.75}	54	58	51	56	52	53	59	54	52	55	-	-	-	-	50	544	11 ml.

All dilutions represent 5 embryos unless otherwise noted by ().

Farm A -- SPAFAS eggs - non-vaccinated flock.

Farm B -- B₁, Newcastle Disease (ND) spray vaccine at 14 days, wing web with MK107 at 14 weeks.

Infectious bronchitis (IB) spray at 3 and 15 weeks.

Farm C -- B₁ ND and IB dust vaccine at 1, 5 and 26 weeks of age.

Farm D -- Dead Newcastle disease vaccine at 15 and 20 weeks of age. IB spray at 6 and 20 weeks of age.

References

1. Brandly, C. A., Moses, H. E., and Jungherr, E. L. Transmission of antiviral activity via the egg and the role of congenital passive immunity to Newcastle disease in chickens. *Am. J. Vet. Res.* 7 (1946): 333-342.
2. Chute, H. L., and Stauffer, D. R. Specific pathogen free poultry flocks in Maine. *Univ. of Maine, Agric. Exp. Sta. Misc. Rep.* 106 (1962): 1-25.
3. Chute, H. L., and O'Meara, D. C. The development of chickens free of common poultry diseases. *Univ. of Maine, Agric. Exp. Sta. Bul.* 613 (1963): 1-23.
4. Cunningham, Charles H. *A laboratory guide in virology.* 1st ed. Burgess Publishing Co., Minneapolis, Minnesota, 1948.
5. Gagliardi, G. Azione del tuorlo incluso nel vaccino pseudo-pestoso inattivato (Effect of the yolk in ND vaccines prepared from embryonated eggs). *Vet. etal.* 11 (1960): 851-867.
6. Keeble, S. A., and Wade, J. A. Inactivated Newcastle disease vaccine. *J. Comp. Path.* 73 (1963): 186-199.

7. Monti, G. Comparazione fra gli anticorpi inibenti la emoa-gglutinazione e gli anticorpe neutralizzanti presenti nel tuorlo di uova di galline vaccinate e operimmunizzate nei confronti della pseudopests. (Comparison between hemagglutination-inhibiting and virus-neutralizing antibodies in egg yolk of fowls vaccinated and hyperimmunized against Newcastle disease). Clinica Veterinaria, Milan, Italy, 77 (1954): 74-78.
8. Nadel, M. K., Fryer, H. C., and Eisenstark, A. The probable minimum numbers of Newcastle disease virus particles required to initiate infection in chick embryos and baby chicks. J. Infect. Dis. 100 (1957): 88-91.
9. Rauscher, Frank J., Reyniers, James A., and Sacksteder, Miriam R. Japanese quail egg embryo as a host for viruses. J. Bacteriol. 84 (1962): 1134-1139.
10. Reed, L. J. and Muench, H. A simple method of estimating fifty per cent end-points. Am. J. Hyg. 27 (1938): 493-497.
11. Upton, Elizabeth, Hanson, R. P., and Brandly, C. A. Antigenic differences among strains of Newcastle disease virus. Proc. Soc. Exp. Biol. and Med. 84 (1953): 691-693.
12. Van Roekel, H., Olesiuk, Olga M. and Beninato, L. P. Symposium on CRD of poultry. III Epizootiology of CRD in chickens. Am.

J. Vet. Res. 19 (1958): 453-463.

13. U. S. D. A., Agr. Res. Adm. B.A.I. The hemagglutination and hemagglutination-inhibition tests for the diagnosis of Newcastle disease. Leaflet, Oct. 21 (1946): 1-4.

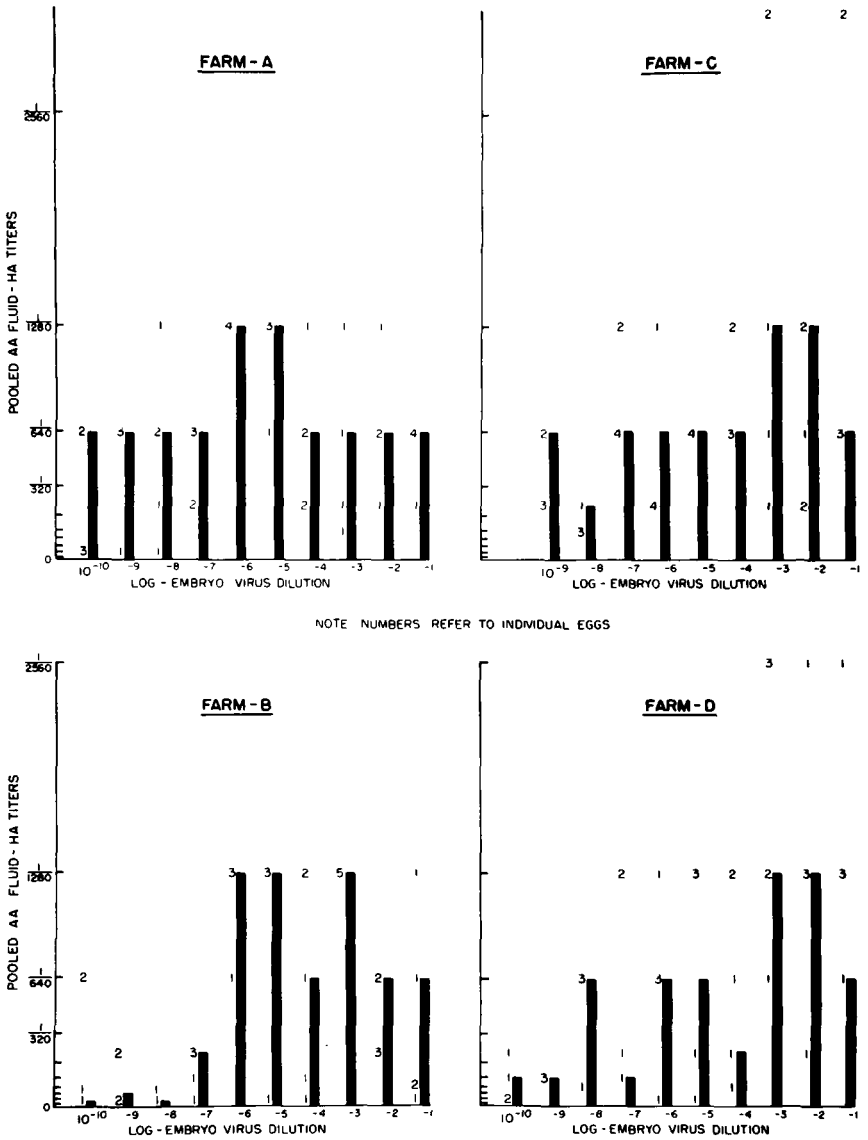
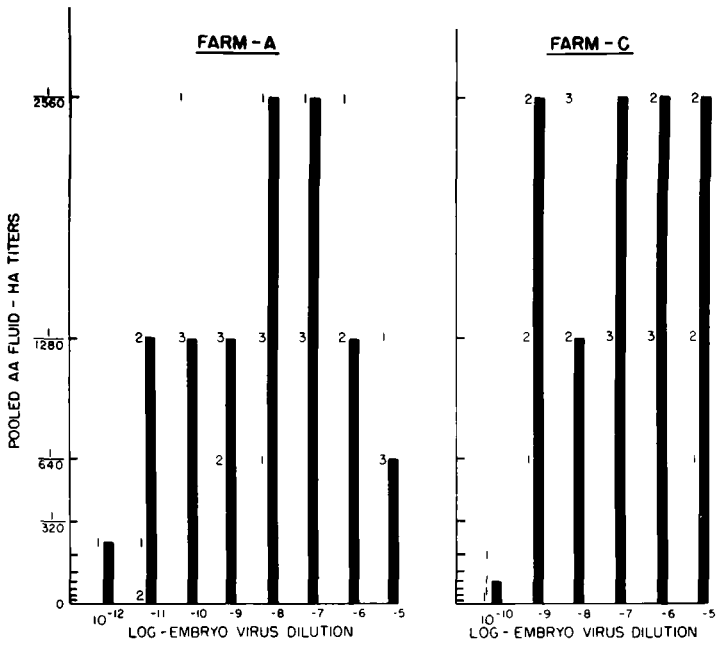


Fig. 1. Newcastle Disease B₁-Virus
 Numbers refer to individual egg HA titers.



NOTE NUMBERS REFER TO INDIVIDUAL EGGS

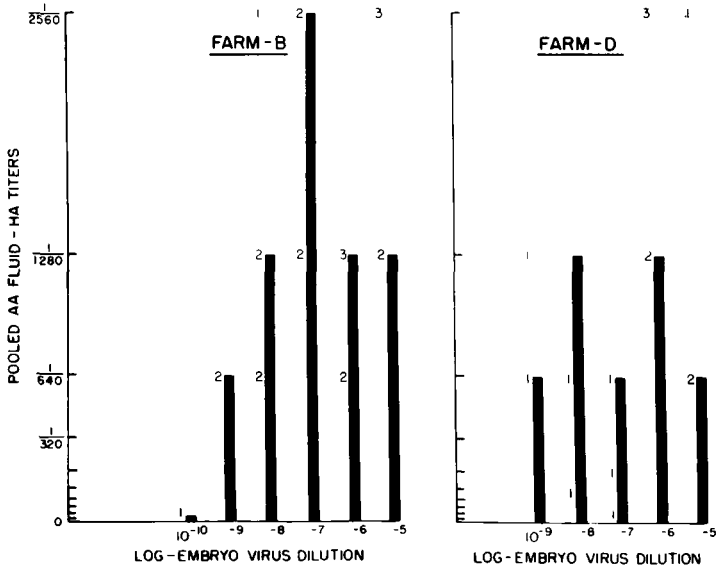


Fig. 2. Newcastle Disease GB-Virus

Numbers refer to individual egg HA titers.