

1950

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### Recommended Citation

Peterson, O. H. and Kerr, K. B. (1950) "Intranasal Newcastle Disease Virus Vaccination of Baby Chicks Infected with *Ascaridia galli*," *Proceedings of the Iowa Academy of Science*: Vol. 57: No. 1 , Article 70.

Available at: <https://scholarworks.uni.edu/pias/vol57/iss1/70>

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## **Intranasal Newcastle Disease Virus Vaccination of Baby Chicks Infected with *Ascaridia galli***

By DR. O. H. PETERSON AND DR. K. B. KERR

An acute infectious disease of poultry manifested by both respiratory and nervous symptoms which occurred in the Dutch East Indies was first described by Kraneveld in 1926 (1 & 2). Shortly thereafter, the disease was observed at Newcastle-on-Tyne in England by Doyle (3) who gave it its present name. In California, Beach (4) described a respiratory-nervous disorder of chickens which he named Avian Pneumoencephalitis and which (5) later was shown to be identical with the disease described by Kraneveld and by Doyle. The disease has been reported from almost all sections of the world. In 1945, Newcastle disease was first identified in the eastern part of the United States and since that time has been reported in each of the forty-eight states.

Newcastle disease is of serious economic importance. Birds of all ages are susceptible to the disease and morbidity approaches 100% when infection occurs in a poultry operation. In the baby chick, mortality is high and losses of 50-70% are not uncommon. In the adult bird, mortality is generally low, however, egg production is seriously affected and usually ceases for a period of ten days to five weeks or more. The first signs of the disease are usually respiratory symptoms and these are frequently followed by nervous manifestations.

Early efforts to develop a satisfactory method of active immunization against Newcastle disease yielded negative or generally unsatisfactory results (2). Among the first vaccines to give promise were those prepared from formalin-treated infected chicken tissues (6 & 7). Later a crystal violet vaccine was prepared from infected chicken embryos and this was found to develop a higher and more uniform virus content than was obtained in the organs of the infected chicken (8). In California, Dr. Beach reported on immunization studies with formalin-inactivated egg-propagated pneumoencephalitis virus (Newcastle) (9). Early attempts to modify the virus by passage in other species such as the pigeon and mouse were unsuccessful (2). Several passages in ducks did not alter the virulence of the virus for chickens (10). Komarov and Goldsmit (11) were unable to change the virulence of the virus for chickens by nine intracerebral passages in pigeons, but found that after eight passages by this route in ducklings the virus became largely non-

pathogenic and produced a substantial immunity in chickens. The first successful modification of Newcastle disease virus by serial passage in embryonating eggs was reported by Iyer and Dobson (12). After thirty-three egg passages in one series and fourteen in another, the virus was virtually non-pathogenic for chickens and yet produced an immunity adequate to protect against challenge with  $10^8$  M.L.D. of virus fourteen to seventeen days later. The virus was not altered in its pathogenicity for embryos after fifty-eight passages.

Beaudette, Bivens, and Miller (13) studied the pathogenicity of one-hundred five strains of Newcastle disease virus on birds four weeks old. It was found that but one strain was of sufficiently low virulence that it could be used to immunize birds of this age without causing undue loss. The vaccines prepared from this strain have been used commercially and, according to Bureau of Animal Industry reports, approximately 100,000,000 doses of live virus vaccine were produced in the United States during the calendar year 1949 (14).

Hitchner and Johnson (15), working at Virginia Polytechnic Institute at Blacksburg, were interested in the possibility of cross-immunization between chick bronchitis and Newcastle disease. In a study of bronchitis virus strains from many investigators, one was received from Dr. F. R. Beaudette of the Rutgers Experimental Station, which apparently produced an immunity to Newcastle disease. This particular strain of virus produced an immunity to Newcastle disease in the chick when given intranasally, but did not produce an immunity when administered by the wing-stab route. Later work established that the strain of virus used for immunization by the intranasal route was not a bronchitis virus but actually was a very mild strain of Newcastle disease virus (16).

Intranasal Newcastle Disease Vaccine is unique among live virus Newcastle Disease Vaccines, in as much as it may be used with safety on day-old chicks and on laying hens without loss of egg production. Other types of live virus vaccine produce a high mortality in day-old chicks and their use is accompanied by an almost complete cessation of egg production when given to laying hens. The wing-stab type of Newcastle disease vaccine should not be used on birds which have other infections as mortality in such cases is usually excessive.

Intranasal Newcastle Disease Vaccine is prepared by inoculating the so-called Hitchner strain of Newcastle disease virus into the allantoic cavity of eleven-day-old embryonated chicken eggs. The

embryos are then incubated for an additional forty-eight hours when the allantoic and amnionic fluids, membranes, and embryo proper are removed, homogenized, frozen, and dried in vacuum from the frozen state. The dry virus material is then pulverized to less than 60-mesh, and sealed in glass vials under vacuum. Immediately before use, the dry vaccine material is reconstituted with normal saline solution.

In the data reported, chicks were raised primarily for future anthelmintic experimentation. Day-old New Hampshire chicks were obtained from an R. O. P. flock maintained in our own laboratory. The White Leghorn chicks originated from a U. S. approved pullorum-clean flock maintained at our own Research Farm. The birds were received as day-old or started chicks, wing-banded, and maintained in standard commercial brooding batteries. The chicks had access to feed and water, ad lib.

Newcastle disease was potentially enzootic during the autumn of 1949 in the building housing the birds because of our other experimental work with Newcastle disease carried out in the same building. Several outbreaks had occurred prior to institution of the routine vaccination procedure. To protect these birds against potential Newcastle disease, the chicks were vaccinated routinely when from one to ten days old with a standard commercial type of Intranasal Newcastle Disease Vaccine prepared at Dr. Salsbury's Laboratories. The vaccine was administered by placing one drop of vaccine in a nostril of the bird.

The brooders were examined daily when feed and water were added, and any dead chicks were removed and their death recorded. Chicks showing nervous symptoms were caught, their band number noted, and a record of the observation was made.

Infective *Ascaridia galli* ova were administered to birds routinely when they were ten days to two weeks old. The estimated infective dose was administered by moistening the feed with a known volume of culture. The culture was diluted with sufficient tap water to ensure a thorough distribution of the eggs when mixed with the feed. No other feed was available to the chicks until this was consumed.

Due to the highly infectious nature of Newcastle disease virus, it is impossible to maintain unvaccinated, Newcastle disease susceptible birds under the potentially enzootic conditions under which the experiment was carried out. Furthermore, the vaccine strain of live virus spreads readily to susceptible stock and produces the mild infection necessary for the establishment of an immunity.

Therefore, the only data available for comparison are mortality statistics obtained during similar seasons of previous years.

The mortality data for the 23 hatches immunized with the intra-nasal vaccine is presented in Parts A and B of Table 1. In all, 1,282 chicks were used with a total death loss from all causes of fifty-eight birds or 4.5 percent. The mean percent mortality for these hatches was  $5.8 \pm 5.7$ . The hatches varied in size from 18 to 206 birds. The death loss varied from zero to six birds, or a variation from 0.0 to 16.7 percent. The latter occurred in the smallest group of birds.

**Table 1**

Mortality Date for Vaccinated Chicks Infected with *Ascaridia galli*

Hatch No.	Date		No. Chicks	No. Deaths to 8 weeks of Age	Per cent Mortality
	Hatched	Vaccinated			
Part A — Data on White Leghorn Chicks					
29	11-22-49	11-22-49	206	4**	1.9
32	12- 6-49	12- 9-49	32	2	6.3
33	12-10-49	12-10-49	206	5	1.9
36	1-14-50	1-17-50	26	1	3.8
37	1-14-50	1-17-50	26	2	7.7
39	1-20-50	1-24-50	30	1	3.3
41	1-27-50	1-27-50	32	1	3.1
			—	—	—
			558 (Total)	16 (Total)	3.9 (Ave.)
Mean mortality $4.0 \pm 3.0$ per cent					
Part B — Data on New Hampshire Chicks					
20	8-24-49	8-30-49	60	0	0.0
21	9- 3-49	9-12-49	22	2	9.1
22	9-24-49	10- 5-49	53	2	3.8
23	10- 1-49	10- 5-49	22	1	4.5
24	10-15-49	11- 3-49	18	3	16.7
25	10-22-49	11- 3-49	27	1	3.7
26	10-29-49	11- 3-49	46	4	8.7
27	11- 7-49	11-10-49	33	4	12.1
28	11-12-49	11-17-49	61	3*	4.9
30	11-27-49	12- 9-49	55	3	5.4
31	12- 6-49	12- 9-49	71	2	2.8
34	12-17-49	12-22-49	23	2	8.7
35	12-24-49	12-29-49	49	2	4.1
38	1-14-50	1-17-50	26	2	7.7
40	1-20-50	1-24-50	77	6	7.8
42	2- 4-50	2- 6-50	81	5	6.2
			—	—	—
			724 (Total)	42 (Total)	4.8 (Ave.)
Mean mortality $6.6 \pm 4.6$ per cent					

\* Two birds demonstrated Newcastle symptoms.

\*\* Two birds demonstrated Newcastle symptoms and recovered.

In attempting to correlate the deaths with the vaccination, it appears that only two of the deaths were directly attributable to Newcastle disease, as judged by time of appearance of symptoms and death. In another hatch (No. 29), two birds developed the nervous symptoms of Newcastle disease but recovered completely.

The mortality data on the White Leghorns is given in Part A of Table 1. In this group, 558 chicks were used with a death loss of 16 or 3.9 percent. The average mortality was 4.0 percent. The mean percent mortality for the Leghorns was  $4.0 \pm 3.0$ . The hatches varied in size from 30 to 206 birds. The death loss varied from one to five or a percentage variation from 1.9 to 7.7. Part B of Table 1 gives the mortality data on New Hampshire chicks. In

**Table 2**

Mortality Data for Unvaccinated New Hampshire Chicks  
Infected with *Ascaridia galli*

Hatch No.	Date Hatched	No. of Chicks	No. Deaths to 8 Weeks of Age	Per cent Mortality
Part A — Data of 1947-48				
1	10-10-47	20	5	25.0
2	10-17-47	18	2	11.1
3	10-24-47	84	0	0.0
4	11- 4-47	66	7	10.4
5	11- 8-47	24	3	12.5
6	1-17-48	121	1	0.08
7	1-24-48	66	7	10.4
8	1-31-48	44	10	22.7
9	2- 7-48	77	13	16.9
		—	—	—
		520 (Total)	48 (Total)	9.2 (Ave.)
Mean mortality $12.1 \pm 11.4$ per cent				
Part B — Data of 1948-49				
10	10-23-48	18	0	0.0
11	11- 2-48	29	1	3.4
12	11-16-48	35	5	14.2
13	11-21-48	71	1	1.4
14	12- 1-48	41	12	29.2
15	12-11-48	52	1	1.9
16	12-21-48	112	1	0.9
17	1- 2-49	48	7	14.5
18	1- 9-49	79	13	16.5
19	1-17-49	69	0	0.0
		—	—	—
		554 (Total)	41 (Total)	7.4 (Ave.)
Mean mortality $8.2 \pm 4.8$ per cent				

this group, 724 chicks were hatched and 35 or an average of 4.8 percent died. The death loss varied from 0 to 6 or a percentage variation from 0.0 to 16.7. The mean percent mortality was  $6.6 \pm 4.6$ .

In comparing the two Parts of Table 1, it is found that a slightly lower mortality occurred in the White Leghorns, a breed which is known to be considerably more susceptible to infection with *Ascaridia* than the New Hampshire breed.

Under routine maintenance of birds for the same type of subsequent experimentation, mortality figures, as given in Table No. 2, Parts A and B, for the two previous years are considered as normal. The periods covered in this table are approximately for the same time of the year as that covered in the data in Table 1. The mortality data for 1947-1948 and 1948-1949, before intranasal Newcastle disease vaccination was available, is presented in Table 2. The mortality statistics from nine hatches in 1947-1948 (Table 2, Part A) show a total loss of 48 out of 520 chickens, or 9.2 percent. The mean mortality for these hatches was  $12.1 \pm 11.4$  percent. The size of the hatches varied from 18 to 121 birds and the mortality varied from 0.0 to 25.0 percent.

Similarly for 1948-1949 (Table 2, Part B), out of 554 birds from ten hatches, 41 died or 7.4 percent. The mean mortality was  $8.2 \pm 4.8$  percent. The size of the hatches varied from 18 to 112 birds and the variation in mortality was from 0.0 to 29.2 percent.

Comparison of the mortality statistics in these two tables reveals that the process of intranasal vaccination with this strain of Newcastle disease virus has no unfavorable influence on the livability of chickens infected with *Ascaridia galli*. An interpretation is not made that the lower mortality in the vaccinated chicks is due to the use of the intranasal vaccination, as there are too many uncontrolled factors involved to permit such a conclusion.

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