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STUDIES ON THE IMMUNOGENIC PROPERTIES OF THREE LENTOGENIC STRAINS OF NEWCASTLE DISEASE VIRUS

by:

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SUMMARY

Chickens with a passive immunity (HI titer 0–1:20) when vaccinated with B1 (Hitchner) strain at 10 days of age, exhibited a slight immunity (HI titer 1:10 – 1:40) 11 days following the first vaccination, but a high immunity (HI titer 1:40 – 1:320) was observed 3 weeks after the second vaccination.

Ninety–eight percent of B1 vaccinated chickens resisted an intramuscular challenge with 10^{-6} ELD 50 of virulent NDV at the age of 12 weeks. However, their related contact controls and challenge controls died within 9 days post challenge. A group of hens that had been vaccinated three times with (TCND) showed high HI and SN titers varying between (1:40–1:1280) and (10^{-2}–10^{-5}) respectively.

The HI and SN titers produced by V2 (Lasota Vaccine, chick–embryo origin) were more than V1 (TCND) and V3 (Lasota tissue culture origin) vaccines. High percentage (96%, 93% & 90%) of the three subgroup hens, when vaccinated with V1, V2 and V3 respectively, were found resistant to contact exposure challenge with 2×10^6 ELD 50 of virulent NDV Zabol strain. The egg production dropped slightly (three to 4%) in vaccinated hens when they were challenged by contact exposure with 2×10^6 ELD 50 of the virulent NDV Zabol strain. In unvaccinated hens the egg production fell more rapidly. They succumbed to ND within a few days. No clinical signs could be seen in chickens vaccinated with B1 (Hitchner) and TCND (Bankowski). However those groups vaccinated with Lasota vaccines (V2–V3) developed signs of a general depression and rales. Three cases of paralysis and one of torticollis were also observed.

INTRODUCTION

Serum-neutralization (SN) and hemagglutination inhibition (HI) tests are most commonly used both for the diagnosis of different diseases and the determination of immunity levels. (2)

In 1952 Levine and Fabricant (11) studied the hemagglutination inhibition response and the degree of resistance to challenge following immunization of chickens with a mesogenic strain of virus by the wing-web stick method of inoculation. They noted that 66–94 percent of the vaccinated birds showed positive hemagglutination-inhibition titer, with a majority having a titer of 1:1280 and higher, and that a great number of the birds resisted the challenge. The immunogenicity of a cell culture adapted virus was studied by Bankowski in 1957 (3) and Bankowski *et al* in 1958. (4)

They found an average hemagglutination-inhibition titer of 1:68 and serum-neutralization of 10^{-7} gradually decreased to 1:50 and 10^{-5} respectively after 5 weeks. The birds were found to resist to challenge when the results were based on the mortality following challenge.

In 1948 Hitchner and Johnson (9) first studied a virus of low virulence suitable for vaccination of chickens by the intransasal route. The immunity conferred following vaccination of one-day-old chickens was reported to persist for a period of 4 months.

Observation by Hitchner (1950) revealed that immunity lasted for at least 19 weeks when chickens were hatched from dams, immunized intranasally by B1 strain.

In 1950 Doll *et al* (8) found that B1 strain induced a better immunity following intranasal inoculation than when it was administered by intramuscular route.

Chickens vaccinated intranasally at 9–30 days of age developed partial immunity in 2 days and a dependable one in 6 days post vaccination. HI titers in chicks, vaccinated intranasally at 5 weeks, varied from 1:40 – 1:640 when tested 12 and 15 days after vaccination thus showing a rapid rise in titer. The immunogenicity of the B1 strain following aerosol inoculation was demonstrated by Johnson and Gross (10) in 1951 and by Hitchner and Rising in 1952.

In 1952 Asplin (1) reported on the immunogenicity of F Strain, a strain isolated from a mild NDV outbreak in England. He found that the strain established a solid immunity for a period of more than one year when chickens were vaccinated either intranasally or intramuscularly at 12 weeks of age.

In 1962 Lancaster (12) observed that F strain would induce an immunity durable for 130 days in one-day-old susceptible chicks after being intranasally inoculated.

Comparative studies were conducted on the immunity of three lentogenic strains of NDV, B1, F and Lasota by Winterfield *et al.* (14) Chickens at 41 weeks of age were given access to a vaccine in the drinking water and challenged three weeks later by intratracheal inoculation. Winterfield and his co-workers found that the Lasota strain had greater spreading potential and produced mild to moderate symptoms, leading to a better immunity than could be obtained by B1 and F Strains.

Comparative studies on the immunogenicity of four lentogenic strains of NDV (TCND, B1, F and Lasota) carried out in our laboratory confirmed that Lasota and F strains conferred slightly better protection than B1 and TCND strains when immunity was challenged with a virulent Newcastle Diseases virus. The Lasota strain caused slightly more respiratory reaction and had a greater spreading potential from chick to chick than F and B1 strains. (13) Serological tests (HI & SN titers) before and after vaccination showed that there was no spreading of TCND vaccine virus to unvaccinated contact controls which were being kept in the same unit. The results were the same as those obtained by Bankowski, R.A. (5-6-7). The aim of this report is to investigate immunogenic properties of three lentogenic strains (B1, TCND and Lasota) of NDV and the significant effects of contact exposure and intramuscular challenge on egg production in vaccinated chickens.

Materials and Methods

Virus:

Three lentogenic and one velogenic strains were used throughout the experiments. The B1 (Hitchner) virus vaccine, with the titer of $10^{8.2}$ ELD 50 /ml, was propagated in embroyonating chicken eggs. The TCND (Bankowski) virus vaccine (V1), originally modified in HeLa cells by Bankowski in 1960, was propagated in lamb kidney cell culture with the titer of $10^{6.2}$ TCID 50/ml., by Tavassoli, A. in 1971. (13)

The NJ Lasota virus vaccine (V2) with the titer of $10^{8.2}$ EID 50/ml. was adapted to and grown in lamb kidney cell culture by Tavassoli in 1971 (V3). The final titer in lamb kidney cell culture was $10^{6.6}$ TCID 50/ml. The velogenic Zabol strain which has been isolated at the Razi Institute by V. Sohrab and M. Baharsefat from natural outbreak of NDV was used as challenge material.

Chickens:

The white leghorn new-born chicks were obtained from the Poultry Department of the Livestock Institute, Tehran. They had not been vaccinated against any disease. The chicks were divided into three main groups- A, B' and C.- and were used in the following manner:

Group A, consisting of 300 ten-day old chicks, was vaccinated intranasally with 500,000 EID 50 of B1 virus vaccine. After being twice vaccinated with B1, they were divided into 4 sub-groups - A1,A2,A3, and A4. Subgroup A1, consisting of 60 chickens, was re-vaccinated 3 times with 0.5 ml of $10^{6.2}$ /0.1 ml. of V1 vaccine.

Subgroup A2 (60 chickens) were inoculated 3 times with 0.5 ml of $10^{6.2}$ 0.1 ml of V2 vaccine. Subgroup A3 (60 chickens) were inoculated 3 times with 0.5 ml. of $10^{6.2}$ 0.1 ml. of V3 vaccine. Subgroup A4, consisting of the remaining 120 chickens in group A, were discarded after being challenged with 1,000,000 doses of Zabol virulent strain of ND virus.

Group B, consisting of 50 chickens, was used to provide contact controls for subgroup A4. They were subsequently challenged and discarded as subgroup A4.

Group C, consisting of 150 unvaccinated chickens, was held in a separate unit and subsequently used as challenge and contact controls. Subgroup C1, consisting of 30 chickens, was used to provide challenge controls for subgroup A4. Subgroup C2 (60 chickens) was used to provide contact controls to determine the spreading potential in chicks experimentally vaccinated with vccines V1, V2 and V3 - i.e. subgroups A1, A2 and A3. Subgroup C3, consisting of the remaining 60 chickens in group C, was used to provide challenge controls for TCND and Lasota vaccine prepared respectively in lamb kidney cell cultures and embryonated eggs.

Experimental Procedures :

All 500 chicks were bled when 8 days old in order to determine their levels of passive immunity as judged by HI and SN titers.

The 300 chicks in group A were vaccinated twice (at 10 and 32 days old) with 500,000 EID 50 of B1 vaccine, which was given intramuscularly. Three weeks after the second vaccination, the HI and SN titers were again determined.

When 12 weeks old, the 120 chicks in subgroup A4 (vaccinated twice with B1 vaccine), the 50 chicks in group B (contact controls) and the 30 chicks of subgroup C1 (challenge controls) were held in a separate unit for challenge with doses of 1,000,000 ELD 50 of virulent ND virus, which was injected intramuscularly.

The remaining 180 chicks of group A (i.e., subgroups A1, A2 and A3 each of which had 60 chicks and had been vaccinated twice with B1) were revaccinated three times with V1, V2 and V3 at 10, 18 and 25 weeks old. The HI and SN titers were determined at the 14th, 26th and 31th weeks of age so as to follow each of three revaccinations.

The three revaccinated subgroups (i.e., A1, A2 and A3) were kept in three separate isolation units, each subgroup together with 20 contact controls selected from group C. These three were later each divided into two parts, each of 30 vaccinated chicks and 10 unvaccinated contact controls. One part of each subgroup was then placed in a separate pen until egg production had stablished at 35 weeks of age. Egg production was maintained under controlled conditions during the 30 days before and after challenge, and all unnecessary handling or disturbance was avoided. Three out of the above 6 parts were challenged intramuscularly with 1,000,000 ELD 50 of virulent Zabol ND virus. The other three parts with the related contact and challenge controls were challenged by contact exposure.

Maternal Immunity in 8-day chicks.

Serum samples from groups A, B and C had HI antibodies ranging in titer from nought to 1:20. Pools of ten sera of these chicks neutralised 10^{-1} ELD 50 of ND virus. No discernible HI or SN titers were found in 50 chicks of group B (unvaccinated controls) and 30 chicks of group C (challenge controls), when these were tested at the age of 30 days. This is set out in Table I.

Response to B1 vaccine in vaccinated chickens.

HI and SN titers in the 300 chicks in group A before and after the 2 vaccinations of B1 are set out in Table I together with the response to intramuscular challenge.

Chicks vaccinated twice with B1 vaccine– at 10 and 32 days old showed significant HI and SN titers when these were determined at the 11th and 53rd day following the respective vaccination, and 1:45 to 1:320 for the second. The SN titers, however, were 10^{-1} to 10^{-2} and 10^{-2} to 10^{-4} respectively. Neither HI nor SN titers were demonstrated in the unvaccinated or the challenge controls when tested at 7½ weeks old.

Of the 120 vaccinated chickens, 117 resisted the intramuscular challenge with 1,000,000 ELD 50 of virulent Zabol ND virus, whereas the 50 contact controls and the 30 challenge controls succumbed nine days after challenge.

Responses of laying hens to TCND & Lasota vaccines.

Results obtained with the three different vaccines are explained and summarised in Tables 2 and 3. It will be seen that higher HI and SN titers were obtained with V2 at 4,8 and 10 weeks post-vaccination than with V1 and V3. All of the 90 chickens comprising half of the three vaccinated subgroups resisted the intramuscular challenge with 1,000,000 ELD 50 of virulent Zabol strain of ND virus. On the other hand, only 96, 93 and 90 per cent of the other halves of the three subgroups resisted challenge or contact exposure. The clinical signs in the birds which resisted the two challenges are listed in Table 3.

As regards the unvaccinated contact controls, two-thirds and one-third of these resisted intramuscular and challenge by contact exposure. All unvaccinted challenge controls succumbed in 9 days.

The rate of egg production in vaccinated hens was very slightly reduced after the challenge- 52.2 per cent before challenge, 47.6 per cent after challenge. These results are listed in Tables 2 and 3.

Discussion

The experiments described in this report demonstrate the degree of immunity of four types of vaccines, prepared by the 3 strains B1, TCND and Lasota of N.D. virus.

The results definitely show that chickens with a passive immunity (from nought to 1:20), when vaccinated with B1 (Hitchner) strain at 10 days of age, induced slight immunity (from 1:10 to 1:40) 11 days after the first vaccination. But at 7.5 weeks of age a higher immunity (1:40 to 1:320) was observed following the second vaccination at 53 days.

98% of B1 vaccinated chickens were resistant to intramuscular challenge with 1,000,000 doses of ELD 50 of virulent Zabol NDV while their related contact controls (50 chickens) and the unvaccinated challenge controls (30) died within 9 days. (Table 1.) Subgroup A1 which had been vaccinated three times with V1 (TCND) showed a high HI and SN titers (1:40 - 1:1280). Table 2.

The HI and SN titers with V2 Lasota vaccine prepared in embryonated chicken eggs were higher than V1 and V3 vaccines (Table 2). There appeared a pause in egg production in vaccinated chickens after each vaccination, after 3 or 4 days, however it resumed its original level. All the three vaccinated subgroups (first part) were resistant to intramuscular challenge with 1,000,000 doses of ELD 50 while 96, 93 and 90% of the other part of vaccinated subgroups resisted the contact exposure challenge with 2,000,000 doses of ELD 50 of virulent Zabol strain NDV.

30 out of 60 contact controls and all of the unvaccinated challenge controls died within 7 days after challenge (Table 2). The average per cent of egg producation in vaccinated subgroup chickens (A1, A2, A3) being 53.3, 51, 52.5 during 30 days prior to challenge dropped to 48.8, 46.9 and 47.3 within a period of 30 days after challenge.

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Table 1: Hemagglutination – Inhbition – (HI) and serum neutralization (SN) titers before and after the 2 vaccinations of 300 Chickens with B1 at 8,21,53 days of age and the chickens's response to intramuscular challenge at 12 weeks of age.

Group N°	Pre-vaccination at 8 days old.		11 days following first vaccination		21 days following second vaccination		N° of chickens challenged at 12 weeks of old	% survival after challenge
	HI	SN	HI	SN	HI	SN		
A 300 chickens vaccinated	(110)[1] - 0 (180) -10 (10) - 20	(20)[2] - 0 10 -10^{-1}	(20) - 10 (92) -20 (188) - 40	9 -10^{-1} 21 -10^{-2}	(70) - 40 (210) -80 (20) - 320	6 -10^{-2} 16 -10^{-3} 8 -10^{-4}	120	98
B 50 chickens contact control	(27) - 0 (20) - 10 (3) - 20	4 - 0 1 -10^{-1}	(32) - 0 (18) - 10	4 -10^{-1} 1 -10^{-1}	(50) - 0	50 - 0	50	0
C 150 chickens contact & challenge control	(99) - 0 (49) - 10 (2) - 20	13 - 0 2 -10^{-1}	(110) - 0 (40) - 10	12 -10^{-1} 3 -10^{-1}	(150) - 0	150 - 0	30	0

* 98 % of vaccinated chickens were resistant to intramuscular challenge with 1,000,000 doses of ELD 50 of virulent Zabol N.D.V.

1– Number in parantheses = Number of sera for HI test = Number of chickens.

2– Number in circles = Number of pools of 10 sera for SN test.

Table 2– Immunological response (HI & SN titers) after 3 revaccinations of the 3 subgroups chickens with TCND & Lasota vaccines propagated in lamb kidney cell cultures and embryonating eggs at 10,18 and 25 weeks of age and rate egg production before and after intramuscular and contact exposure challenge-s at 40 weeks of age.

Subgroup N°	Vaccines	4 Weeks following third vaccination		8 Weeks following fourth vaccination		10 Weeks following fifth vaccination		Average % egg production 30 days before challenge	% Survival after challenge at 40 weeks old.		Average % egg production 30 days after challenge	Clinical signs following intramuscular and cont.Expo. challenge
		HI	SN	HI	SN	HI	SN		IM(1)	cont.Exp*		
A_1 60 chickens	V_1	40-320	$10^{-2}-10^{-4}$	80-640	$10^{-3}-10^{-5}$	40-1280	$10^{-4}-10^{-5}$	53.3	100	96	48.8	19 Cases of depression.3 cases of Respiratory sign
A_2 60 chickens	V_2	40-640	$10^{-3}-10^{-4}$	80-1280	$10^{-2}-10^{-5}$	160-2560	$10^{-3}-10^{-6}$	51.0	100	93	46.9	13 Cases of depression & rales. 5 cases of Resp. & nervous signs.
A_3 60 chickens	V_3	40-320	$10^{-2}-10^{-4}$	40-640	$10^{-2}-10^{-5}$	80-1280	$10^{-3}-10^{-5}$	52.5	100	90	47.3	23 Cases of dep. & rales. 7 cases of resp & nervous signs.
Contact controls	-	0-10	$0-10^{-1}$	10-20	$0-10^{-1}$	10-40	$10^{-1}-10^{-2}$	-	20	10	-	-
Challenge controls	-	0	0	0	0	0	0	-	0	0	-	-

1=IM=Intramuscular.

* The contact exposure challenge was done by introducing into each pen of the A1,A2 & A3 second parts. 4 N. D. susceptible birds previously injected, with 2 ml. of virulent zabol N.D.V. strain (2000,000 ELD 50) by intratracheal route.

Table 3- Clinical signs in chickens vaccinated with B1, TCND and Lasota vaccines at 10 and 45 weeks of age following intramuscular and contact exposure challenge.

Challenge	Number of Chickens	Group and Subgroup	Vaccines	Clinical signs
Intramuscular	120	A	B1	7 Cases: depression and rales. 5 Cases: respiratory signs, paralysis.
	30	A_1	TCND (V_1)	3 Cases: depression
	30	A_2	Lasota (V_2)	3 Cases: depression and rales 2 Cases: severe respiratory signs, paralysis.
	30	A_3	Lasota (V_3)	4 Cases: depression and rales. 2 Cases: severe respiratory signs.
Contact exposure	30	A_1	TCND (V_1)	16 Cases: depression and rales. 4 Cases: respiratory signs & death
	30	A_2	Lasota (V_2)	17 Cases: depression and rales. 6 Cases: respiratory signs, torticollis and death.
	30	A_3	Lasota (V_3)	23 Cases: depression and rales. 13 Cases: respiratory and rales, torti collis, death.