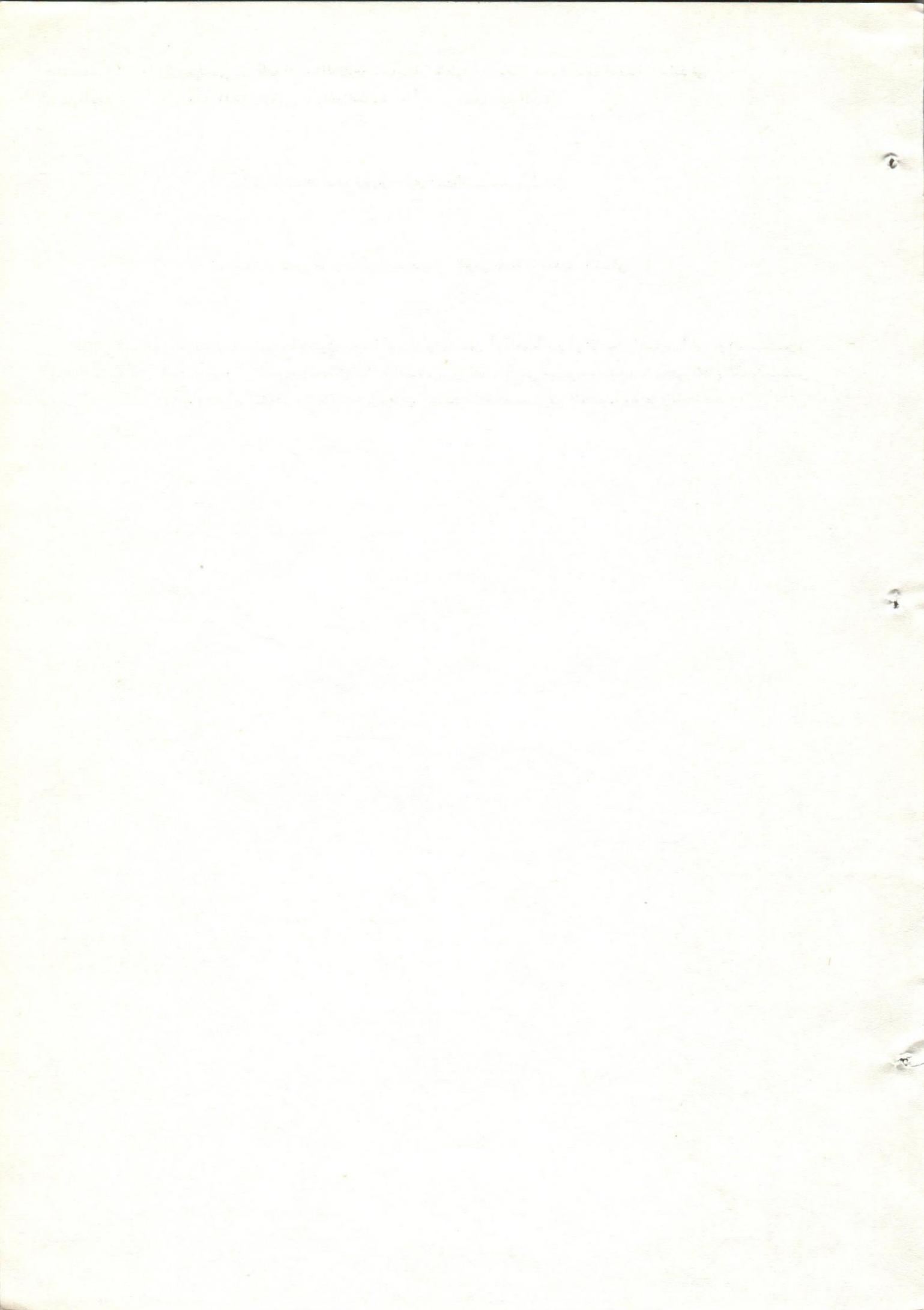


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مطولات تثبيط عترة فيروس مرض النيوكاسل

أ. سناء ، سلمى صابر ، نرجس برهومة ، شوقى نديم ، سعيد الصبان

فيروس النيوكاسل الحشرى الضارى عندما عومل بمادة البيتا بروجيولاكتون أو الفورمالين أو الاثيل اثيلين أمين أو الحرارة وذلك لكى يفقد ضراوته ، وجد أن الفيروس المعامل بمادة البيتا بروجيولاكتون أعطى أحسن صناعة عندما أعطى كلقاح للدواجن
وبداسة هذا اللقاح وجد أن الكتاكيت المحصنة بجرعتين أعطيت مناعة أحسن من المحصنة بجرعة واحدة فقط .



TRIAL OF DIFFERENT INACTIVATORS FOR THE VISCEROTROPIC VELOGENIC NEWCASTLE DISEASE VIRUS

(With 4 Tables)

By
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SUMMARY

A viscerotropic velogenic Newcastle disease virus was inactivated by BPL, formalin, EEI & heat. The BPL inactivated vaccine gave the best protection results.

Using one&two doses of vaccine the two doses gave better immunity.

INTRODUCTION & REVIEW OF LITERATURE

The world history of Newcastle disease (ND) showed that once it became established in a country or region, it tended to become endemic (DOYLE, 1948, LANCASTER, 1963 a). In such situation the ND should be eradicated or controlled. The control measures had tended to be of an emergency nature involving the use of vaccination programs against the disease. In Egypt the vaccines first used were the live attenuated komerov strain vaccine for 2 months old chicks to be given intramuscularly and the F strain vaccine for days old chicks intraocularly.

Beside the living vaccines, also inactivated vaccines had been studied since more than 30 years (BAUDETTE, 1943).

The immunogenicity of inactivated ND vaccine was variable depending on the inactivating agent. DOYLE & WRIGHT (1950) used crystal violet, ethylene glycol as inactivators, and reported that immunity was established at 7 days after vaccination & persisted for at least 12 months. DOLL *et al.* (1951) observed that all chickens vaccinated with single or multiple injections of a formalin inactivated virus were susceptible to respiratory route. HANSON *et al.* (1951) compared the immunogenicity of 5 strains of NDV in a formalized vaccine. They found that certain strains were much better immunologically than others, but no relation between immunogenicity & virulence or heat stability of the strain.

Recently beta propiolactone (BPL) had come into use as an inactivating agent. Mackard Chotisen (1956) used BPL inactivated NDV and studied the response in chickens after vaccination. GILL *et al.* (1959) studied the effect of 3 different adjuvants on the immunogenicity of the (GB) Texas strain of the virus. Several workers reported that the inactivated vaccine induced satisfactory immunity comparable or even superior to live vaccines. (HANSON *et al.* 1951; KOCH, 1959; FABRICANT, 1956; SCHMIDT, 1959; LEVINE, 1962; APPLETON *et al.* 1963; LOMBARDI, 1966; BOX and FURMINGER, 1959).

A variety of methods had been used for inactivation of NDV vaccine including chemicals, heat & ultraviolet irradiation. A review of these methods was given by LANCASTER (1966). Among chemicals formalin & BPL had been extensively used with different adjuvants and adsorbants were utilized to enhance and prolong the immune response.

The aim of this experiment is to carry out a comparative study on various chemicals to be used for inactivation of NDV as formalin, BPL, Ethyl Ethylen Imine (EEI), in the preparation of inactivated ND vaccine.

MATERIALS & METHODS

Eggs:

Fertile eggs were purchased from the General Poultry Company. They were incubated and used at 9-10 days old.

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ND Virus:

A field local strain was isolated from a severe outbreak of ND. The strain was lyophilized, and characterized proved to be viscerotropic velogenic strain (VVNDV) (SHEBLE and REDA 1979). This strain was used for vaccine preparation and for challenging experiments.

Chickens:

500 day old chicks coming from immune mothers were purchased from the General Poultry Company. They were kept in an isolated place until used.

Preparation of the vaccine:Cultivation of Virus.

The cultivation technic was carried out according to ALLAN *et al.* (1973). One thousand eggs were inoculated into the allantoic sac with 0.1 ml./egg of 10^{-5} diluted seed virus. Eggs that died after 48 hrs were kept in the refrigerator until harvestion. The harvested fluid was centrifuged at 3000 r.p.m. and tested for sterility, HA titre & ELD₅₀ in chick embryos, were determined. The infective AF fluid was concentrated by dialysis against polyethylene glycol to about 10 folds. The concentrated fluid was tested for sterility & its HA & ELD₅₀ were determined.

The Inactivation of the Virus.a - With BPL.

This was carried out according to SCHMIDT & LNETTAL (1967). Equal volume of 0.4% BPL solution & virus suspension were mixed in a magnetic stirrer at 37°C for 2 hrs to yield a final concentration of 0.2% of BPL. The pH of the treated material was 7.2 and was stored at 4°C until tested.

b - With Formalin.

Mix equal volumes of concentrated virus and diluted formalin in a magnetic stirrer at 37°C for 2 hours to yield a final concentration of 0.05%. The vaccine was stored at 4°C until tested.

c - With Ethyle Ethylin Imine (E.E.I.):

This was carried out by mixing equal volume of diluted 0.1% E.E.I. in hank's solution and virus suspension were then mixed in a magnetic stirrer at 37°C for 2 hours to yield a final concentration of 0.05% of E.E.I. The vaccine was stored at 4°C until tested.

d - Inactivation by Heat.

The virus was inactivated in a water bath at 58°C for ½ hr. and vaccine was then stored at 4°C until tested.

EXPERIMENTAL:

The chicks were divided into groups of 50 each. Blood samples were collected from all chicks before inoculation & tested for HI and freedom of HI antibodies. Two separate experiments were carried out. In experiment (1) each group of 50 chicks were divided into two equal numbers each of 25 chicks and each group was given a certain vaccine. 25 birds were inoculated with the vaccine concentrated & 25 with the vaccine diluted 1:10. In the first experiment the chicks were given two doses, one at 2 weeks of age & a second booster dose at 5 weeks. Each chick received 1 ml. intramuscularly. In the second experiment the chicks were given only one dose at 8 weeks. At various time as 1, 2, 3, & 4 weeks after vaccination blood samples were collected from 10 birds of each group tested for HI test. After 4 weeks of vaccination each bird with the controls were challenged giving 1 million doses of the viscerotropic velogenic local strain intramuscularly. The results are shown in table 1 & 2 for the single dose experiment and in tables 3 & 4 for the double doses.

RESULTS

The harvested embryonic fluid for vaccine preparation proved to be bacteriologically sterile before and after concentration and its HA titer 1:640 and chick embryo titer gave $10^{-8.5}$ /1 ml. before concentration and HA titer became 1:1024 and chick embryo titer was $10^{-9.5}$ /1 ml. after concentration. Safety tests in 9 day old embryonated chicken eggs for each vaccine and in susceptible 8 week chicks, proved complete inactivation of the virus in each vaccine.

Giving one dose of vaccine as shown in table (1), the HI titer showed a rise in using all the 4 inactivators,

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the BPL gave a better geometric mean titer of 4.4 at 1st week and 5.09 at the 4th week, with the 1:10 dilution of vaccine and (5.25 & 2) with the concentrated, then the formalin which gave (3.9 & 3) with 1:10 & (4.00 & 3.3) with the concentrated. The lowest titers were given with heat as it ranged between (2.6 & 1.27) & (3.16 & 2). giving 2 doses of vaccine, the BPL also gave the best titers of 4.6 at the 1st week and 3.9 on the 4th, then the EEI which showed (4.7 & 3.64) and (5.2 & 4.06). The least titers were given by formalin which were (3.6 & 2.6) and (3.15 & 2.9) successively.

Regarding the results of challenge in the single dose as shown in table 2, the BPL gave a high percentage of protection of 72.8%, but formalin although gave 33.3% protection, the concentrated gave 88.8% also the EEI gave 42.8% with the 1:10 dilution & 75% with the concentrated vaccine.

The heat treated vaccine also gave the lowest percentage of protection of 40 & 55.5%. Giving 2 doses of vaccine as shown in table 4. the percentage of protection was the highest in the BPL whether in 1:10 dilution or the concentrated vaccine which gave 80 & 92%, then comes the EEI giving 83 & 88.8% and the least was the formalin that gave 70 & 75% successively.

From these results it could be concluded that the BPL gave in general the best results and that the two doses of vaccine gave better immunity than one dose.

DISCUSSION

In controlling ND by vaccination of young chicks was difficult because of maternal immunity (LANCASTER, 1968; STONE & BONEY, 1968; HIGGINS, 1971; ALLAN, 1974) and the variability in level of antibodies in commercial chicks (BONEY & STONE, 1970; ZAKEY, RONES & LEVY, 1973). The administration to young chicks under 3 weeks of age when given the inactivated vaccine with a lentogenic strain gave better immunity than giving the inactivated vaccine alone (ZAKAY-RONES & LEVY, 1973; PICAULT *et al.* 1975; QUAGLIS *et al.* 1975; LOMBARIDI *et al.* 1975).

In this experiment comparing the effect of 3 chemical inactivators on the VVNDV and giving a single or double doses the BPL treated vaccine gave the best protection & two doses of vaccine gave higher immunity than one dose. These results were in agreement with ALLAN *et al.* (1973). BOX *et al.* (1968) vaccinated a group of chickens with BPL inactivated vaccine at 3 weeks and 3 weeks or 3, 9 & 16 weeks of age challenge revealed that 88% of birds given a single dose at 3 weeks were immune at 20 weeks of age & 75% at 26 weeks & 83% of birds given 2 doses were immune at 26 weeks as were 92% of these given 3 doses.

KEEBLE and CAID (1962) injected chicks 2 - 4 weeks old intramuscularly with BPL vaccine adsorbed on aluminium hydroxide and standardized to contain 50 chick protective dose (0.5 ml.). The vaccine conferred immunity to challenge 9 - 12 weeks after vaccination.

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Table 1 : Geometric Mean of HI Titer of Susceptible Chickens Vaccinated By Single Dose of Inactivated VVND Vaccine Using 4 Different Inactivators

Type of Inactivator	Dilution of Vaccine	Geometric mean HI Titer in Weeks After Vaccination			
		1st Week	2nd Week	3rd Week	4th Week
EPL	1 : 10	4.4	4.8	5.5	5.09
EPL	Concent.	4.0	4.8	4.09	3.6
Formalin	1 : 10	2.9	3.16	4.25	3.00
Formalin	Concent.	4.00	3.16	2.2	3.3
EKI	1 : 10	4.6	4.5	2.5	1.57
EKI	Concent.	5.25	4.7	3.75	2.00
Heat	1 : 10	2.6	2.6	3.7	1.27
Heat	Concent.	3.16	3.09	2.5	2.00

EPL = Betapropiolactone
EKI = Ethyle Ethylene Imine .

Table 2 : Results of Challenge of Susceptible Chickens Vaccinated by Single Dose of Inactivated VVND Vaccine Using 4 Different Inactivators

Type of Inactivator	Dilution of Vaccine	Results of Challenge		
		No. of Birds Vaccin	No. Survived the challenge	% of protection
EPL	1 : 10	22	16	72.8%
EPL	Concent.	22	16	72.8%
Formalin	1 : 10	18	6	33.3%
Formalin	Concent.	18	16	88.8%
EKI	1 : 10	14	6	42.8%
EKI	Concent.	16	12	75 %
Heat	1 : 10	20	8	40 %
Heat	Concent.	18	10	55.5%
Controls		20	0	0%

EPL = Betapropiolactone
EKI = Ethyle Ethylene Imine
g = Each chick was given one million doses of VVNDV .

Table 3 : Geometric Mean HI Titer of Susceptible Chickens Vaccinated by Two Doses of Inactivated VVND Vaccine Using three Different Inactivators

Type of Inactivator	Dilution of Vaccine	Geometric Mean HI Titer in Weeks After Vaccination			
		1st Week	2nd Week	3rd Week	4th Week
EPL	1 : 10	4.6	4.8	4.58	3.9
EPL	Concent.	5.25	4.4	4.7	4.17
Formalin	1 : 10	3.6	3.2	2.8	2.6
Formalin	Concent.	3.15	3.6	3	2.9
EKI	1 : 10	4.7	4.5	4.13	3.64
EKI	Concent.	5.2	4.7	4.07	4.06

EPL = Betapropiolactone
EKI = Ethyle Ethylene Imine .

Table 4 : Results of Challenge of Susceptible Chickens Vaccinated by Double Dose of Inactivated VVND Vaccine Using 4 Different Inactivators

Type of Inactivator	Dilution of Vaccine	Results of Challenge		
		No. of Birds Vaccin	No. Survived the challenge	% of protection
EPL	1 : 10	10	9	90 %
EPL	Concent.	12	11	92 %
Formalin	1 : 10	10	7	70 %
Formalin	Concent.	12	9	75 %
EKI	1 : 10	12	10	83 %
EKI	Concent.	18	16	88.8%
Controls	Non vaccinated	20	0	0 %

EPL = Betapropiolactone
EKI = Ethyle Ethylene Imine

