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**TRIALS FOR PRODUCTION OF ALUMINIUM HYDROXIDE GEL
AND OILY ADJUVANT INACTIVATED NEWCASTLE DISEASE
VACCINES, IN COMPARISON WITH COMMERCIAL
IMPORTED OILY VACCINE**
(With 5 Table & 1 Fig.)

By

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محاولة لتحضير لقاح النيوكاسل الميت الزيتي والمعلق في هيدروكسيد
الألومنيوم ومقارنته باللقاح الزيتي المستورد

نادية حسن ، انصاف خشبة ، فكرية البرديني ، سلوى الأصيلي ، الهام الأبياري ،
صلاح وهبة ، نرجس برهومة

تم انتاج لقاح النيوكاسل الميت الزيتي والمعلق في هيدروكسيد الألومنيوم وقد تمت
مقارنته باللقاح الزيتي الميت المستورد . وقد تم التقييم بناء على اجراء اختباري
منع التلازن الدموي واختبار التحدي وكانت النتائج كالآتي : - تم استخدام عترة
متشنر (ب) المستضعفة الحية في انتاج اللقاح الميت للنيوكاسل حيث أثبتت النتائج
أنها أفضل العترات الحية للنيوكاسل وكانت القوة العياريية لها ٩٠.٧ مل / ٠ وكان
القوة العياريية باستخدام اختبار منع التلازن الدموي هي لوج ٧ - لوحظ أن مدة ١٨ ساعة
هي أفضل فترة زمنية يتم عندها التثبيط بعد اضافة مادة الفورمالين بتخفيف ١ : ١٠٠٠ -
وجد أن اضافة مادة هيدروكسيد الألومنيوم أو مادة المستحضر الزيتي إلى اللقاح ليس له
أي رد فعل غير مرغوب فيه في الكتاكيت المحصنة بعد فترة ملاحظة أسبوعان بـ
التحصين ، كما أظهرت النتائج أن ليس هناك أي فرق بين استخدام اللقاح المحللي
حيث أظهرت نتائج اختبار مدى الاحتفاظ بالفاعلية Keeping Quality Test حماية
الكتاكيت خلال فترة ٦ أشهر من التحصين بكل من اللقاحين - أظهرت النتائج أن النسبة
المثوية للغة المناعية في الكتاكيت المحصنة بلقاح النيوكاسل الميت المعلق في
هيدروكسيد الألومنيوم كانت في الشهر الأول ٩٢٪ وفي الشهر الثاني ٨٢٪ أما في
الشهر الثالث فكانت ٧٩٪ وقلت النسبة المثوية للغة المناعية في الكتاكيت إلى
٧٠٪ ، ٦٥٪ ، ٦٠٪ في الشهر الرابع والخامس والسادس على التوالي .

SUMMARY

Inactivated Newcastle disease virus (NDV) vaccines were prepared in aluminium gel [A1(OH)₃] and oil emulsion (OE) adjuvants and compared with a commercially produced oil adjuvant vaccine. The lentogenic Hitchner B₁ strain was chosen as the best virus seed, giving an allantoic fluid titre of $10^{10.5}$ /ml. egg infective dose₅₀ and 7 log₂ haemagglutination titre. Virus inactivation was completed at 18 hours with final concentration of formalin at 1:1000. Both oil emulsion and aluminium gel vaccines were safe and immunogenic. The OE vaccine prepared from local oils had a high viscosity and was unsuitable for field use.

Both OE and A1(OH)₃ vaccines gave 80% protection for 6 months, with geometric mean haemagglutination inhibition (HI) titers of 3.5 and 2.8 respectively with the A1 (OH)₃, the protection percent was 93, 83 and 79 for the first, second and third month respectively. The protection percent dropped to 70, 65 and 60 in the fourth, fifth and sixth month respectively.

INTRODUCTION

The control of NDV is carried out by vaccination with either live or inactivated ND vaccines. Vaccine efficacy depend on many variables, such as the nature and the amount of antigen administered and the presence of adjuvants to enhance immunogenicity, manipulation of such variable can improve vaccines.

PIERCY, et al. (1964) produced the first A1(OH)₃ adsorped vaccine for NDV. This vaccine was extensively used until OE inactivated vaccines superceded them (ALLAN, 1972; PHILLIPS, 1973).

There was extensive use of the OE vaccines during the epizootic in the United Kingdom in 1970, where such vaccines were very effective. Other countries in Europe confirmed their value (ZANELLA, 1966), first by serological demonstration of the extremely high levels of antibodies they produced and later by freedom of vaccinated birds from disease and any drop in egg production.

This work was undertaken to validate the use of A1(OH)₃ inactivated vaccine, to examine the use of local oils for an OE vaccine and compare these with the commercial product.

MATERIAL and METHODS

I - Virus strains:

a) Vaccinal strains:

1. Seed strains of Komarov, HB₁ and "F" strains were originally supplied by the Poultry Vaccine Research Laboratory at Weybridge, and were used to grow up virus stock.
2. LaSota strain of NDV and OE inactivated vaccines were imported by a commercial Company.

b) Virulent strains

Velogenic viscerotropic NDV (VVNDV) strain was locally isolated and identified by SHEBLE and REDA (1967).

II- Embryonated chicken eggs and chickens:

1. Commercial 9-11 day old embryonated chicken eggs. These were obtained from a commercial source, and the parent stock had been vaccinated against NDV.
2. One day old chicks were supplied from the same source of eggs and held in isolation to be used as susceptible chickens.

III- Chemicals:

- Sodium hydroxide and aluminium potassium sulphate were supplied by El-Nast Pharmaceuticals.
- A formaldehyde solution 37-41% was obtained from BDH Limited Poole, England.
- Resila (a white paraffin oil) was obtained locally and also purified lanolin.

METHODS:

II- Titration and serological methods:

These were carried out according to the standard methods described in the FAO publication (1978).

II- Preparation of alhydrogel:

The preparation of 10 liters involved two stages, first aluminium potassium sulphate 1.08 kg was dissolved in 8.4 litre of distilled water at 40°C. Then 0.27 grams of sodium hydroxide was dissolved in 1.8 litre distilled water, 60 ml was discarded and then mixed with the first solution. A basket centrifuge was used to wash the gel to remove excess NaOH and an equivalent amount of distilled water was added to replace the fluid removed.

EXPERIMENTAL METHODS:

I - To determine the best NDV strain for preparation of the virus antigen: Hitchner B₁, "F", Komarov, LaSota and a virulent strain of NDV were inoculated by the allantoic route to 9-10 days embryonated chicken eggs, 4 days later, allantoic fluid were harvested and assayed for virus (Methods for examination of poultry biologies, 1965).

II- Inactivation:

Formalin was added as a 1:10 dilution at a final concentration of 1:1000 to a bulk container, in which the allantoic fluid contents were stirred at 37°C. Care was taken to transfer the contents to the second container and ensure that no virus was splashed onto the neck of the vessel and escape inactivation. The inactivation continued for 24 hours period and fluid samples were collected after 10, 14, 18 and 24 hrs. Each sample was diluted 1:10 and 1:100 and inoculated into 9-10 days old embryonated eggs. These were harvested after 4 days and two blind passages were carried out. The allantoamniotic fluid (AAF) were assayed for haemagglutinin, (HA) after the 2nd and 3rd passages (Methods for examination of poultry biologies, 1965).

III- Vaccine preparation:

- OE vaccine was prepared by mixing equal volumes of an aqueous phase of AAF with oil, consisting of 80% Risela and 20% Lanolin. This was homogenized to prepare stable emulsion (Methods for examination of poultry biologies, 1965).
- The 2% Aluminium gel was mixed with an equal volume of inactivated AAF and dispensed after thorough stirring.

IV- Safety tests:

Seventy, one week old chickens were inoculated as below:

- 1 - 10 x 0.2 ml. A1(OH₃) by subcutaneous (S/C) route in the neck.
10 x 0.2 ml. A1(OH₃) by intramuscular (I/M) route in the thigh.
- 2 - Similar inoculations were made both the local and commercial OE vaccines.
- 3 - 10 chicks were kept as controls.

The chickens were observed for 2 weeks for local or systemic reactions. After 5 weeks they were killed and post mortem examination were made on each bird.

V - Potency test:

One hundred chicks were kept and tested for haemagglutination inhibition (HIT) antibody at 20 days of age (all were negative). Groups of 20 were inoculated I/M with 0.5 ml. of the three vaccines, A1(OH₃), OE local and commercial one. After 21 days blood samples were collected from all chickens including the uninoculated controls

and they were challenged with the viscerotropic velogenic strain of NDV. Each chicken received 0.5 ml. containing $10^{5.5}$ EID₅₀. All the chickens were observed for 14 days and morbidity and mortality was recorded which was considered specific for NDV after 3 days post challenge.

VI- Keeping quality:

The local A1(OH₁) and commercial OE vaccines were held at 4°C for 6 months. Aliquots were taken for the inoculation of chickens and efficacy was determined by HAI titers and resistance to challenge.

VII- Duration of immunity:

One hundred chicks were vaccinated with the local A1(OH₁) and held for 6 months. Every month, 15 birds were bled for assay of HAI and 15 birds were challenged, together with 10 unvaccinated birds with the velogenic strain, and observed as above.

RESULTS

The local oils which were used to prepare an emulsion without the addition of any emulsifying agents, produced a very high viscous product. This was extremely difficult to inoculate and only potency tests were carried out (Table III). It was not considered suitable for field use. Table (1) shows the determination of virus titers with the different candidate NDV strains. This shows that "F" and HB₁ strains gave the highest titer of the virus in the AAF and were considered to be the best strain for the virus vaccine production. Table (2) gives details of the inactivation process. The local A1(OH₁) and OE together with the commercial OE vaccines were used to compare the potency of vaccines (Table III), while the A1(OH₁) and the commercial OE were tested for the keeping quality (Table VI). The duration of immunity was tested only with A1(OH₁) (Table II and Fig. 1).

DISCUSSION

Since many years, live virus ND vaccine have been used to a greater extent than inactivated one. Recently years improvement have been made in the preparation of ND inactivated vaccines by the use of better adjuvants.

Lentogenic Hitchner B₁ & "F" strains of NDV are the best vaccinal strains used to prepare the inactivated vaccine. They gave a titer of $10^{10.5}$ /ml EID₅₀ of the allantoic fluid, and a log₂ 7 HA titer (Table 1). The use of lentogenic strain for preparation of inactivated vaccines was suggested by GOUGH, *et al.* (1977) who showed that NDV grows to a higher titer if lentogenic virus was used as seed.

The inactivation time for the HB₁ or "F" strain were 18 hours using a formalin in a final concentration of 1:1000 (Table II). GARLIC and AVERY (1976) suggested that the use of formalin as an inactivating agent may give a more stable vaccine than that use BPL.

Both oily and A1(OH₃) vaccine proved to be safe for chicken since no local or systemic reactions were seen in any bird after 2 weeks observation period, but there was a major constraint in production of OE vaccine using the local oils. These resulted in difficulty of preparing a stable water-in-oil emulsion with low viscosity. Later on we will try again the addition of oil phase emulsifiers to improve the quality of OE vaccine.

Vaccinated birds, whether receiving OE adjuvant or A1(OH₃) adsorbed vaccines were found to be protected against I/M challenge with VVNDV 21 days post-vaccination (Table III).

The keeping quality of A1(OH₃) and that of commercial OE vaccines were measured by protection percent and log₂ HI titer at monthly intervals for 6 months. During that period the vaccine proved potent, giving protection percentage of 80% for both vaccines at the end of 6 months. The geometric mean titer of (HAI) antibody persisted at a higher level and together with OE than with the A1(OH₃), this may be due to the slow release of antigen from its oily adjuvant. In contrast the A1(OH₃) gave a higher titer earlier than OE and this may be due to its watery nature being adsorbed quicker than the oily one, giving higher HI titer, then decline quicker than the oily one (Table VI).

CHEDID (1985) reported that, the role of adjuvant such as A1(OH₃) or OE is primarily sequestering the antigen and maintaining it in the inoculation site, thus allowing a slow release in the blood and lymphocyte circulation.

Duration of immunity of A1(OH₃) was evaluated by protection percentage and HAI titer during 6 months. Table (V) showed that A1(OH₃) gave protection for 6 months although the HAI titers were declining over this period, birds even with a low HAI titer withstood challenge.

The A1(OH₃) is suitable for broilers and layers in the growing period and specially as a booster vaccine to give a high anamnestic response after primary live virus vaccination. Clearly the OE vaccines are marginally better than the A1(OH₃) and efforts will be made to improve the local OE vaccines by the addition of aqueous and oil phase emulsifying agents.

REFERENCES

- Allan, W.H. (1972): Newcastle disease control. Agricultural, London, 79 (10): 413-420.
- Chedid, (1985): Adjuvants of immunity. Ann Inst. Pasteur Immunol., 138 D. 283-289.
- FAO, Animal Production and Health (1978): Newcastle disease vaccine, their production and use. Series No. 10.
- Garlic, B. and Avery, R.J. (1976): Inactivation of Newcastle disease by B-Propiolactone (brief, 1976 report) Archives of Virology, 52 (1/2): 175-179.
- Gough, R.E.; Allan, W.H. and Nedelcin, D. (1977): Immune response to monovalent and bivalent Newcastle disease and infectious brochitis inactivated vaccine. Avian Path., 6 (2): 131-142.
- Methods for examination of poultry biologics (1965): National Academy of Science. National Research Council - Washington, D.C.
- Piercy, S.E.; Macleod, A.H. and Blaxland, J.D. (1964): Duration of immunity trials with beta-propiolactone inactivated Newcastle disease virus vaccines. Vet. Res., 76: 1084-1089.
- Philips, J.M. (1973): Vaccination against ND: An assessment of haemagglutination inhibition titers obtained from field samples. Vet. Rec., 93 (22): 577-583.
- Sheble, A. and Reda, I. (1967): Cited by Khaphagy, A.K. (1972): M.V.Sc. Thesis, Faculty of Vet. Med., Cairo University.
- Zanella, A. (1966): Further studies on the immunizing activity of inactivated vaccines in oily vehicle. Clinica Vet. Milano, 89: 391-396.

Table (I)

The results of the EID₅₀ of allantoic fluid harvests from different NDV candidate strains and the log₂ HA they produced

Type of NDV strains	Infectivity titer/ ml. EID ₅₀	Log ₂ HA titer
Velogenic viscerotropic	8.3	4
Komarov	9.5	5
LaSota	10.0	6
Hitchner B ₁	10.5	7
"F" strain	10.5	7

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Table (II)

Results of completion of inactivation in hours

Virus isolation	Hours post addition of formalin							
	10		14		18		24	
	No. of +ve HA /Total tested	Inactivation %	No. of +ve HA /Total tested	Inactivation %	No. of +ve HA /Total tested	Inactivation %	No. of +ve HA /Total tested	Inactivation %
Origin	8/10	20	2/10	80	0/10	100	0/10	100
1:10	5/10	50	0/10	100	0/10	100	0/10	100
1:100	1/10	90	0/10	100	0/10	100	0/10	100

Table (III)

Potency test of local Al(OH₃) ,OE vaccines and commercial OE inactivated NDVThe geometric mean of log₂ HI titer and the protection%

Kind of the vaccine	No. of tested birds	Geometric mean HI titer	Mortalities/ Total tested	Protection %
Local Al(OH ₃)	20	5.8	3/20	85
Local OE	20	4.3	2/20	90
Commercial OE	20	5.2	1/20	95
Non-vaccinated control	10	0	9/10	10

Table (IV)

The keeping quality of local $Al(OH)_3$ & commercial OE inactivated vaccines as measured by protection percentage and \log_2 HI titer

The haemagglutination HI titer of broiler chickens vaccinated with local $Al(OH)_3$ and commercial OE inactivated vaccines during storage at 4°C

for 6 months

Duration at monthly intervals Type of tested vaccine	1		2		3		4		5		6	
	HI GMT	Protection %	HI GMT	Protection %	HI GMT	Protection %	HI GMT	Protection %	HI GMT	Protection %	HI GMT	Protection %
Local $Al(OH)_3$ inactivated vaccine	5.5	100	5.0	90	4.5	90	4.0	83	3.0	83	2.8	80
Commercial OE inactivated vaccine	5.1	90	6.0	90	5.0	88	4.4	85	4.0	80	3.5	80
Un-vaccinated control	0	5	-	10	-	10	-	15	-	10	-	15

* HI GMT : Haemagglutination inhibition geometric mean titer

Table (V)
Duration of immunity of Al(OH₃) at monthly intervals for
6 months post-vaccination

Haemagglutination inhibition titer and protection percent
induced by vaccinated chickens with Al(OH₃) at monthly
intervals for 6 months post-vaccination

Parameters	Duration of immunity at monthly intervals post-vaccination					
	1	2	3	4	5	6
HI GMT (log ₂)	5.1	4.2	3.5	3.2	3.0	1.2
Protection% of vaccinates	93	83	79	70	65	60
Protection % of controls	0	0	5	5	10	10

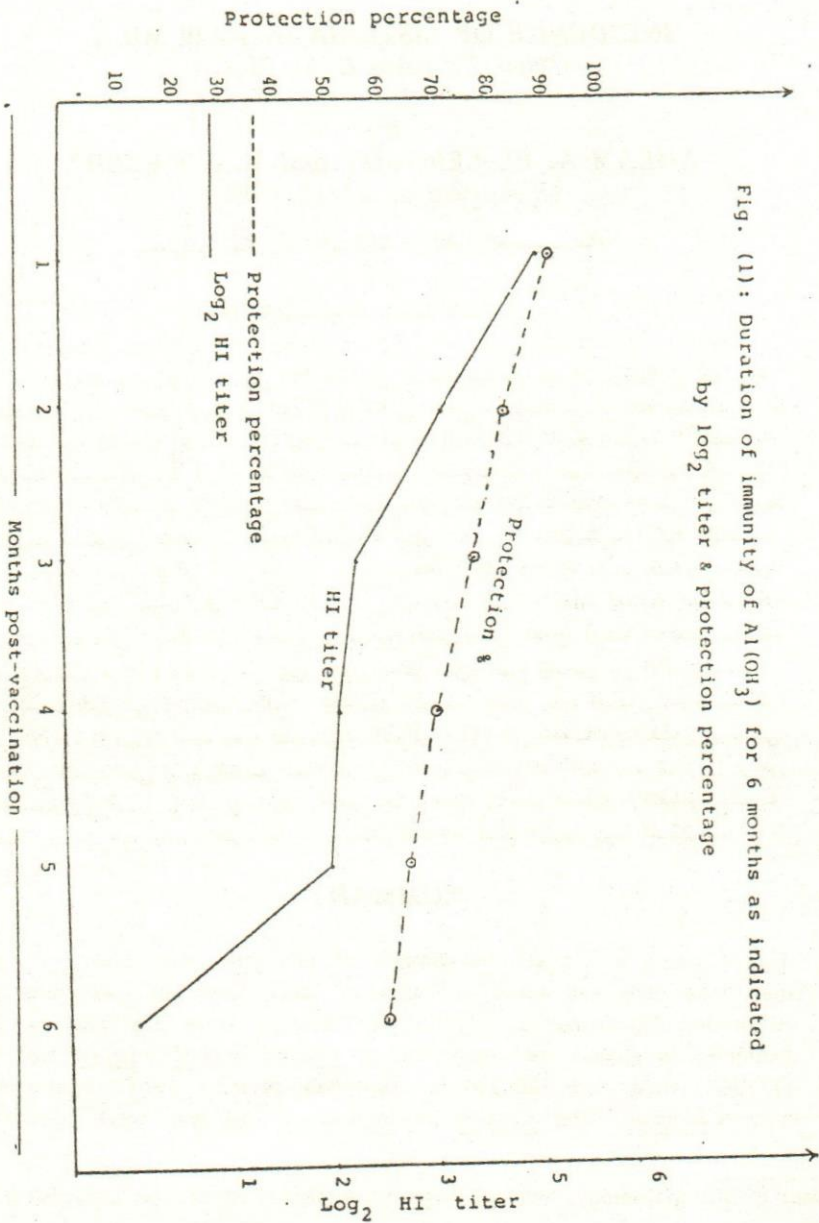


Fig. (1): Duration of immunity of Al(OH₃) for 6 months as indicated by log₂ titer & protection percentage