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# PREPARATION AND EVALUATION OF KILLED NDV VACCINE USING NEW OIL ADJUVANT

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## SUMMARY

Preparation of inactivated oil Newcastle disease virus (NDV) vaccine using different new oil adjuvants Montonide ISA 70 VG, ISA 763A VG and ISA 775 as oil adjuvants in comparison with paraffin oil was carried out. The prepared vaccines with Montanide ISA 70 VG, 763 A VG and 775 VG induced high antibody titers than that induced by vaccines prepared with paraffin oil when determined by serological tests (HI and SNT). The highest titer observed in prepared vaccine by ISA 70 VG oil adjuvant after 3 weeks post vaccination and remained high till 12 weeks post vaccination and has long duration of immunity reached to 12 month with a protective antibody titer and protection against challenge while prepared vaccine by Montonide ISA 763 A VG oil adjuvant reached 10 month with Montanide ISA 775 reached to 9 months, but in paraffin protective antibody titer reached only till 5 month .

## INTRODUCTION

Newcastle disease virus (NDV) is one from virus poultry pathogens that severely endangering out poultry industry. The virus is a highly contagious septicaemic fatal and destructive disease which attacks chiefly chickens and turkeys. The first to discover this virus was in Newcastle, England after the initial report about the disease in Java, Indonesia by Kranaveld, (1926).

Oil emulsion adjuvant is particularly suitable vaccine that will be required to give long term pro-

tection. The antigen released slowly and provides long term stimulation the immune system. Gupta et al.,(1993).

Sitj'a ñBobadilla et al.,(2008) reported that Montanide ISA 763A is based on non-mineral oils and was prepared as oil-in-water emulsion for better tolerance. Montanide adjuvant stimulates different innate immune factors and clearly enhances the production of specific antibodies. The result was better for the antigen ñMontanide formulation, which also showed negligible undesired side effect and a good correlation between antibody level and challenge test. The observed difference in response to the assayed formulations could be useful for further development of immunoprophylactic therapies. In our present studies, the new Montanide ISA 70 VG, Montanide ISA 763 A VG and Montanide ISA 775 VG oil adjuvants were used in preparation of NDV inactivated oil emulsion vaccines, and compared them with the paraffin oil adjuvant which used conventionally used in the preparation of NDV inactivated oil emulsion vaccines.

## MATERIALS AND METHODS

### 1. Viral strain:

A-Newcastle disease virus La Sota strain: It was supplied by central laboratory, Weybrige; England its titer was 1010.5 EID 50/ml and was used for preparation NDV vaccine.

B-Newcastle disease virus (virulent strains): it is a field local isolate, was obtained from the Newcastle Department, Veterinary Serum And Vaccine Research Institute, Abbassia, Cairo (VSVRI) with infectivity titer 106 EID50 /dose..

### 2. Chicken Eggs:

Specific pathogen free (SPF) embryonated chicken eggs (9-10 days old ) were obtained from SPF production farm, Koum Osheim, Fayoum, Egypt and were used for propagation ,titration and assurance of complete inactivation of prepared batch of NDV vaccine.

### 3. Chickens:

Seven hundreds and fifty one day old chicks were obtained from united company for poultry the birds were kept under strict hygienic measures in isolated and disinfected cages till 21 days

### 4. Adjuvants:

4.1Oil adjuvant ISA 70 VG, 763 A VG and 775 VG:

They were obtained from Seppic, Comestics, Pharmacy Division, Paris, France. A ratio of adjuvant to the antigen was done according to manufacturer instructions.

4.2 Paraffin oil:

Paraffin oil (white oil) MICBIL, Alexandria Whiterex.

4.2. A .Emulsifiers:

- Sorbitan monooleate (span 80) supplied by Ubichem LTD.
- Polyethylene sorbitan (tween 80) supplied by Sigma Company, USA.

**5. Propagation, titration and inactivation of NDV vaccine:**

NDV was propagated in 10 day old SPF eggs according to Allan et al. ,(1973) titrated by the method described in OIE, (2004), its titer was $10^{11.3}$ EID50 /ml then inactivation by formalin at a final concentration of 0.1% for 18 hours.

**6. Preparation of NDV vaccines:**

6.1. The vaccines prepared using Montanide ISA 70 VG,763 A VG and 775 VG :

Mixing of antigen together with of Montanide ISA 70VG, 763 A VG, 775 VG respectively (30/70) (v/v) according to manufacturing instructions.

6.2. Preparation of inactivated NDV vaccine using paraffin oil:

It was prepared according to Stone et al.,(1990) that the virus with tween 80 is making aqueous phase. Aqueous phase was emulsified in oil phase by ratio 1:3.

**7. Experimental Design:**

A total no of seven hundred and fifty, 21 day old chicks were divided into three groups.

**Group (1):** Consisted of 500 chicks which in turn divided into 5 groups each of 100 birds, four were vaccinated with different vaccines and one was kept as unvaccinated control .Ten birds from each group were subjected to potency test (challenge test).

**Group (2):** Consisted of 100 chicks were used for detecting of keeping quality at 6 and 12 months.

**Group(3):** A group of 150 chickens were divided into five groups each of 30 chickens used for detection of PD50 for different oil emulsions Montanide ISA-70 VG, 763 A VG, 775 Paraffin and unvaccinated control.

**8. Quality control of prepared vaccines:**

Quality control including purity, sterility and safety was carried out according to Code of American Federal Regulation, (1985).

**9. Characterization of the vaccines:**

For evaluation of the emulsification process of vaccines drop test, emulsion viscosity and emulsion stability were done according to Becher, (1965) and Cessi and Nordelli, (1973), respectively.

**10. Efficiency of the prepared vaccines:**

This was carried out using the following serological tests:

10.1 Haemagglutination inhibition test (HI):

The test was performed for determining the haemagglutination antibodies against NDV virus

according to the standard procedure described by Majujabe and Hitchner, (1977).

10.2 Virus neutralization test (SNT):

It was carried out to estimate neutralizing antibodies against NDV according to the method described by Rossiter et al., (1985).

10.3 Challenge test :

Ten chickens of the vaccinated and control groups were challenged with virulent NDV intramuscularly at 21 days post vaccination.

## RESULTS

### Serological tests:

Table (1) compared results of HI mean antibody titers induced by different adjuvants test had higher mean antibody titers in groups of chicks inoculated with prepared vaccines by Montanide ISA 70 VG oil adjuvant (13.5)$\log_2$ and SNT was ( 8.0 ) $\log_2$ three weeks post vaccination.

While in case of groups of chicks inoculated with prepared vaccines by Montanide ISA 763A VG oil adjuvant (10.0) $\log_2$ with a mean antibody $\log_2$ titer of SNT (6.25).when the group of chicks inoculated with vaccine prepared by Mon-

**Table (1): Results of Potency Test Showing Antibody Titers determined By HI & SNT**

| Type Of adjuvants used in Vaccine Preparation | Type Of adjuvants used in Vaccine preparation | Time | Mean antibody titer of HI test ($\log_2$) | Mean antibody titer of SNT test ($\log_2$) | Protection Rate% |
|---|---|---|---|---|---|
| ISA 70VG | ISA 70VG | 3WPC | 13.5 | 8.0 | 100% |
| | | 1WPC | 8.8 | 6.1 | |
| ISA7763AVG | ISA7763AVG | 3WPC | 10.0 | 6.25 | 100% |
| | | 1WPC | 7.5 | 6.0 | |
| ISA775VG | ISA775VG | 3WPC | 9.7 | 5.0 | 100% |
| | | 1WPC | 8.0 | 4.3 | |
| Parafin Oil | Parafin Oil | 3WPC | 9.0 | 5.0 | 90% |
| | | 1WPC | 7.0 | 5.0 | |
| Unvaccinated Control | Unvaccinated Control | 3WPC | 0 | 0 | 0% |
| | | 1WPC | 0 | 0 | |

WPC=Weeks post challenge
WPV=Weeks post vaccination
HI=Heamagglutination Inhibition test
SNT= Serum Neutralization test

tanide ISA 775 VG oil adjuvant was tested HI titer was (9.7) $\log_2$ and SNT mean antibody titers was (5.0) $\log_2$ while in case of paraffin oil antibody titers determined by HI test had lower titer in groups of chicks inoculated with prepared vaccines by oil adjuvant (9.0) log 2 and mean titer of SNT was (5.0) $\log_2$ .

**Challenge test:**

Three weeks post vaccination, ten birds from each group of vaccinated birds were challenged each bird was given 1ml /intramuscular route of vvNDV ($10^{6.5}$EID50/ml) via intramuscular route. The inoculated birds were kept under observation for 15 days and were examined daily for any clinical signs appeared . Results showed that all prepared vaccines are potent and safe but paraffin oil adjuvanted NDV vaccine was less potent.

**Duration of immunity:**

Determination of duration of immunity antibody titers through out HI and SNT after vaccination as shown in table (2) and (3). The highest titer obtained by the different types of vaccines were 3 week post vaccination and the protective cut off protection of antibody titer remained until 11th month in the prepared vaccines with Montanide ISA 70 VG, 10th month with Montanide ISA763 A VG, 9th months with Montanide ISA 775 VG and 5th month with paraffin.

**Keeping quality:**

When chickens were vaccinated with NDV oil adjuvant Montanide ISA70 VG, ISA763 A VG, ISA 775 VG which were stored vaccines at 4°C. vaccines by were safe, potent for 12 month but Paraffin oil were safe and potent after storage 6 month .

**Determination of $PD_{50}$:**

Determination of the protective dose fifty for NDV in different oil adjuvants by inoculation of chickens with different oil adjuvant NDV vaccines at different doses, full dose (0.5 ml), half dose (0.25 ml) and 1/4 dose (0.125 ml) Montanide ISA 70 VG showed that, These doses induced a protective rate of as follows: 100%,90% and 100% respectively. Montanide ISA 763 A VG 100%, 100% and 90% respectively.

Montanide ISA 775 VG 100%, 100% and 80% respectively. Paraffin oil adjuvant 90% 60% and 30% The calculated for paraffin oil was $PD_{50}$ = 0.199 ml.

*Table (2):shows the duration of immunity of different types of inactivated NDV vaccine using different oil adjuvants estimated by Haemagglutination Inhibition test. ($log_2$ anibody titer)*

| Type of adjuvants used in Vaccine preparation | Weeks Post Vaccination | | | | | | | | | | | Months Post Vaccination | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| ISA 70 VG | 7.75 | 11.2 | 13.5 | 12.9 | 12.1 | 11.6 | 11.2 | 10.59 | 10.39 | 10.0 | 9.8 | 9.8 | 8.8 | 8.9 | 8.3 | 7.5 | 6.4 | 5.8 | 5.0 | 4.3 | 4.0 | |
| ISA 763 A VG | 6.0 | 9.6 | 10.0 | 9.8 | 9.7 | 9.3 | 9.1 | 8.8 | 8.5 | 8.38 | 8.07 | 7.5 | 6.8 | 6.8 | 6.5 | 5.3 | 5.0 | 4.8 | 4.3 | 3.3 | 2.1 | |
| ISA 775 VG | 4.5 | 9.05 | 9.7 | 9.65 | 9.11 | 8.85 | 8.3 | 7.2 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 6.9 | 6.2 | 5.9 | 5.6 | 4.8 | 4.1 | 3.5 | 3.0 | |
| Paraffin Oil | 4.1 | 8.0 | 9.0 | 8.5 | 8.0 | 8.0 | 7.5 | 7.0 | 6.9 | 6.4 | 6.0 | 5.6 | 5.0 | 4.6 | 3.4 | 3.1 | 2.6 | 0 | 0 | 0 | 0 | |
| Unvaccinated Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

N.B.:Haemagglutination inhibition test titers after only one vaccination at 3 weeks of age

Table (3): Shows The Duration of Immunity of Different Types of Inactivated NDV Vaccine Using Different Oil Adjuvants Estimated By Serum Neutralization Test($log_2$ anibody titer)

| Type Of Adjuvants Used In Vaccine Preparation | Weeks Post Vaccination | | | | | | | | | | | Months Post Vaccination | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | |
| ISA 70 VG | 3.8 | 6.0 | 8.0 | 7.2 | 7.0 | 6.9 | 6.8 | 6.5 | 6.3 | 6.2 | 6.0 | 6.0 | 6.0 | 5.9 | 5.8 | 5.0 | 5.0 | 5.0 | 4.0 | 3.8 | 3.5 | | |
| ISA 763 A VG | 2.7 | 3.0 | 6.25 | 8.0 | 7.7 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 6.5 | 6.5 | 6.5 | 6.0 | 6.0 | 5.0 | 4.5 | 4.0 | 3.0 | | |
| ISA 775 VG | 3.0 | 4.5 | 5.0 | 6.5 | 6.5 | 6.3 | 6.1 | 5.9 | 5.5 | 5.2 | 5.1 | 5.0 | 4.8 | 4.6 | 4.3 | 4.3 | 4.1 | 4.0 | 3.8 | 3.5 | 3.0 | | |
| Paraffin Oil | 2 | 2 | 5 | 6 | 7 | 7 | 6 | 5 | 5 | 5 | 4 | 3 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Unvaccinated Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |

N.B.:Serum neutralization test titers after only one vaccination at 3 weeks of age

## DISCUSSION

Vaccination is the most effective method to control and prevent ND in poultry. Inactivated oil emulsion vaccines generally result in local inflammation at site of injection. Mineral oil also persists in tissues and there is a possibility of contamination by carcinogenic aromatic hydrocarbons Yamanaka et al., (1993).

The progress in vaccination is directed towards the selection of the proper adjuvant that can elaborate high and long lasting immunity. Seppic , (2002).

The previous results revealed that the prepared Montanide ISA 70 VG, 763 A VG, 775 VG oil adjuvant vaccines were more safe and were considered the best vaccine adjuvant as compared with paraffin oil adjuvants. These results agreed with Abd El-hady, (2001).

From the above mentioned results, the superiority of new oil adjuvant Montanide ISA 70 VG, Montanide ISA 763 A VG and Montanide ISA 775 VG inactivated vaccines in the maintenance of high level of antibody for several months were attributed to these immunostimulant effect as well as they act by depot formation. These groups of Montanide ISA, forms a stable water in oil droplets intended to give slow release of antigen at site of injection Aucouturier et al., (2001).

Montanide ISA 70 and ISA 775 have the ability to elicit strong humeral response and improve protection against challenge Aucotuier et al (2002). Montanide ISA 763 A VG adjuvant stimulate different innate immune factors and clearly enhance the production of specific antibodies Sitjá- Bobadilla et al., (2008).

Interaction between cell membrane and surfactant take place and facilitate /modification antigen uptake by APC. Aucotuier and Ganne, (2002).

Lymphocyte trapping and modification of cell membranes, emulsions is the result of oil phase draw together the antigen and APC.

In conclusion the best oil adjuvant with low viscosity, long duration of immunity, gives the highest titers of antibody and with low tissue reaction is Montanide ISA 70 VG.

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# تحضير وتقييم لقاح ميت لفيروس مرض النيوكاسل باستخدام مساعد زيتي جديد

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*** لمعمل المركزي للرقابة على المستحضرات الحيوية ، العباسية – مصر .

تم عمل اختبار السمية لزيت مونتانويد ISA70VG وكذلك ISA763AVG٧٧٥ VG، ISAالجديد على ذكور الفئران السويسرية وكانت النتيجة سلبية.وتم تثبيط فيروس النيوكاسل تثبيطاً كاملاً بعد ١٨ ساعة من بداية التثبيط.

تم استخدام زيوت جديدة هىISA 775 VG & ISA 763 A VG, ISA70 VG في تحضير لقاح ميت للفيروس المسبب لمرض النيوكاسل ومقارنتها باللقاح المحلي المحضر بزيت البرافين.وتم عمل اختبار الأمان للقاحات المحضرة ودلت النتائج على أن اللقاح المثبط ضد مرض النيوكاسل المحضر بواسطة زيت اى اس ايه ٧٠ وكذلك زيت اى اس ايه ٧٦٣، ٧٧٥ كان أكثر أماناً مع عدم وجود أي آثار في مكان الحقن.

تم تقييم اللقاحات المحضر بواسطة ثلاث اختبارات سيرولوجية وهي اختبار مانع التلازن الدموي واختبار التعادل واختبار الاجار الترسيبي على سيرم مجموعات الدجاج المحصنة باللقاحات ودلت النتائج على أن نسبة الأجسام المناعية المضادة كانت عند الأسبوع الثالث واستمرت حتي الأسبوع الثاني عشر والأسبوع السابع والسادس في مجموعات الدجاج المحصنة باللقاحات المحضرة بواسطة زيت اى اس ايه ٧٠، ٧٦٣، ٧٧٥ على التوالي وعند مقارنتها باللقاح المحضر بالبرافين كانت أعلى نسبة أجسام مناعية متكونة في الأسبوع الثالث وانخفضت تدريجياً ابتداء من الأسبوع السادس.

وكذلك دلت النتائج على استمرارية كفاءة التحصين بواسطة اللقاح المحضر بواسطة زيت اى اس ايهVG ٧٠ حتى الشهر الحادي عشر والشهر العاشر عند استخدام اللقاح المحضر بواسطة زيت أي اس ايه ٧٦٣، ٧٧٥. وعند مقارنتهم باللقاح المحضر باستخدام البرافين كان حتى الشهر السادس بعد الحقن.وعند حفظ اللقاحات عند درجة حرارة ٤م دلت النتائج على أن اللقاح المحضر بـــ أى اس ايه ٧٠ استمر صالح للاستخدام حتى ١٢ شهر كذلك اللقاحات المحضرة بـــ اى اس ايه ٧٦٣، ٧٧٥ والبرافين استمرت صالحة للاستخدام حتى الشهر الثامن.

تم تحديد الجرعة اللازمة من اللقاحات المحضرة من لقاح النيوكاسل المثبط عن طريق استخدام الزيوت المختلفة لحماية ٥٠% من الطيور المحصنة بالحقن في أربعة مجاميع من الدجاج لكل مجموعة بجرعات مختلفة (٠,٥، ٠,٢٥، ٠,١٢٥) عند عمر ٣ أسابيع وبعد ثلاثة أسابيع من الحقن تم عمل اختبار التحدي المناعي بالعترة الضارية للنيوكاسل وكانت النتائج جميعها جيدة وتعطي نسبة حماية توضح أمان وكفاءة اللقاحات النيوكاسل المحضرة باستخدام المساعدات الزيتية الجديدة.