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Preparation and Evaluation of a Combined Inactivated Oil Emulsion Vaccine against Newcastle and Chicken Necrotic Enteritis Diseases

Salman, O. G. A.*; Hanaa, A. Mostafa; Ghada, M. Sadek**; Hussein, A. S.*; Elham, A. El-Ebiary**; Khodeir, M. H.* and Makharita, M. A.****

*Veterinary Serum and Vaccine Research Institute; Abassia, Cairo
**Central Laboratory for Evaluation of Veterinary Biologics

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SUMMARY

The present work represents a trial for preparation and evaluation of a combined vaccine against Newcastle disease (ND) and necrotic enteritis (NE) as a viral and bacterial diseases affecting poultry industry in a dramatic form. The obtained results showed that the prepared vaccine was stable; free from foreign contaminants; safe and immunogenic for vaccinated chickens. The comparison between the efficacy of single ND and NE vaccines with the efficacy of the prepared combined ND and NE vaccine revealed that both vaccines were immunogenic inducing protection against the challenge with virulent strain of ND virus of 87 and 93% in single and combined vaccines respectively. Non vaccinated chickens were unable to withstand the virulent strain of ND virus. Haemagglutination inhibition test (HI) for estimation of ND antibodies was confirmed by the results of challenge test. It was noticed that the combined vaccine induced higher antibody titer and higher protection than those induced by single vaccine. On the other hand, using serum neutralization test for estimation of the antibodies against NE revealed that the antibody titer was higher in combined vaccine than single vaccine. So, it could be suggested that the combined ND and NE vaccine is preferable than the single vaccines.

INTRODUCTION

Newcastle disease (ND) and Necrotic Enteritis (NE) represent a viral and a bacterial disease respectively affecting poultry industry in a dramatic form.

Newcastle disease (ND) is one of the oldest infectious diseases affecting many domestic and wild avian species but it is most notable in domestic poultry due to their high susceptibility and the potential for sever impacts of an endemic on poultry industries.

The caused virus of ND was categorized on the bases of its pathogenicity and virulence into lentogenic, mesogenic and velogenic viruses (Beard and Hanson, 1984). Clinical signs of ND ranged widely from subclinical to per-acute forms with sudden death according to the pathogenicity of the affected virus strain. The disease control depends mainly on administration of the specific potent vaccines by the suitable route at the suitable age (Westbury, 1984).

On the other side, Necrotic Enteritis (NE) represents an acute enterotoxaemic disease causing massive losses among broilers. Clostridium Perfringens type A and to lesser extent type C have been reported to be the causative agent (Kaldhusdal and Lovland, 2000; Engstrom *et al,* 2003 and Van Immerseel *et al,* 2004). The disease affects primarily broilers of 2-5 weeks old raised on litters but can also affect commercial layers pullets raised in cages (Broussard *et al,* 1986). The disease may be present as acute clinical disease (CNE) or subclinical disease (SNE). The clinical signs of CNE are usually very short and often the only sign is sudden death with increasing mortality and persists in the flock for 5-10 days with mortality rate up to 50% (Merck and Co, 2006). The incidence of NE is effectively controlled through out the use of antibiotics as food additives and growth promoters; however their outright ban in Europe, as well as consumer cancers in North America had led to an increased interest in for control of intestinal pathogens (Wikie *et al,* 2006). A specific vaccine was produced for the first time in Egypt by Hussein *et al* (2007) using a local strain of Cl. Perfringens type A in two formulae, aluminum hydroxide gel and oil adjuvant vaccines. Such vaccines were found to be safe and potent providing good protection for vaccinated broilers.

The present work aims to provide a double protection for chicken against two of devastating diseases; ND and NE; affecting them in a dramatic form saving time and efforts.

MATERIALS AND METHODS

1-Newcastle Disease virus (NDV):

1.1-Lasota virus strain was kindly supplied by the Central Veterinary Laboratory, Weighbridge England. The virus titer was $10^{10.5}$ EID_{50}/ml and its HA units was 2^{11}. It was used for preparation of experimental single and combined vaccine batches. NDV was propagated in specific pathogen free (SPF) embryonated chicken eggs (ECE) according to **Allan et al (1973).**

1.2- Velogenic viscerotropic NDV used for challenge and its titer was $10^{6}EID_{50}$/ml.

2-Clostredium Perfringens:

Local isolate of toxigenic Cl. Perfringens type A was supplied by the Department of Anaerobic Bacterial vaccine

Research, VSVRI. It was used for preparation of experimental single and combined vaccine batches according Hussein *et al* (2007) using clarified and concentrated toxoid by ultra filtration system.

3- Chickens:

Two hundreds specific pathogen free (SPF) chickens of 2 weeks old were supplied by the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Abassia, Cairo. One hundred chickens were used in the safety test and one hundred chickens were used in the potency test.

4-Mice:

Weaned Swiss albino mice of 18-20 gm body weight, were supplied by the Department of Pet Animal Vaccine Research, VSVRI for estimation of NE antibodies using serum neutralization test (by using stander anti toxin and toxin).

5-Vaccine preparations:

5.1-Single ND vaccine:

Inactivated double oil emulsified ND vaccine was prepared according to Stone *et al* (1978).

5.2- Single NE vaccine:

Double oil emulsified NE vaccine was prepared using clarified concentrated toxoid according to Hussein *et al* (2007).

5.3- Combined ND and NE vaccine:

Firstly, the protein contents of the inactivated ND virus suspension and NE toxoid were estimated according to Ohnishi and Barr (1978) where the protein content was 40mg/ml for ND virus and 136mg/ml for NE toxoid. Two equal volumes of inactivated ND virus suspension and NE toxoid were mixed thoroughly then adjuvanted with white mineral oil using double emulsification method (water in oil in water) according to Stone *et al* (1978).

6- Quality control of the prepared experimental vaccine batches:

6.1-Physical properties:

6.1.1-Emulsion stability:

According to Becher (1965) drop test was carried out where 2 drops of emulsion were placed separately on a clean glass slide and each drop was mixed either with a drop of oil or a drop of water. A water in oil in water emulsion blend readily with oil but not with water.

6.2- Sterility test:

Testing the freedom of the prepared experimental vaccine batches from foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma) was carried out following the British Veterinary Codex (1970) in the Central Laboratory for Evaluation of Veterinary Biologics, Abassia, Cairo.

6.3- Safety test:

According to British Veterinary Codex (1970) the safety of each prepared vaccine was tested through inoculation of the double dose (1ml) at the same time through the subcutaneous route and another time through the intramuscular route in each group of 25

SPF chickens of 2 weeks old. A chicken group of 25 birds was kept without inoculation as test control. All chickens were kept under daily clinical examination for 14 days.

6.4-Potency test:

Each vaccine batch (single ND, single NE and combined ND&NE vaccines) was inoculated in each group of 25 SPF chickens of 2 weeks old through the subcutaneous route using a dose of 0.5ml administrated twice with 2 weeks interval. In addition, a group of non-vaccinated 25 chickens was kept as control. Each chicken group divided in 2 subgroups and housed separately under hygienic measures receiving balance ration and adequate water. Serum samples were obtained from each chicken group for estimation of the induced antibody titers using haemagglutination inhibition (HI) test for ND and serum neutralization in mice for NE after two weeks from second dose. On the 4th week post vaccination vaccinated chickens with the single and combined ND and NE vaccines were challenged against the virulent ND virus while the second subgroup of vaccinated (ND+NE) was subjected for antibody titer till the 7th week post second vaccination.

7-Haeagglutination inhibition test (HI):

It was carried out to estimate ND antibodies in vaccinated chickens according to Westbury (1984).

8- Serum neutralization test (SNT):

SNT was carried out in mice to estimate NE antibodies in vaccinated chickens according to European Pharmacopeia (2001).

RESULTS AND DISCUSSION

Table (1): Mean Newcastle haemagglutination inhibition antibody titers in different vaccinated chicken groups

Used vaccine	Mean ND-HI antibody titer (log2/ml)/ weeks post vaccination								
	0	1 WPV*	2 WPV	3 WPV	4 WPV	5 WPV	6 WPV	7 WPV	8 WPV
Single ND	0	2	3	4	5	6	6	7	7
Combined (ND&NE)	0	2	4	5	6	7	8	8	8
Control	0	0	0	0	0	0	0	0	0

*WPV= week post vaccination

Table (2): Mean serum neutralizing NE antibodies in vaccinated chickens

Used vaccine	Mean NE neutralizing antibody titer (IU/ml) 2weeks post the second vaccination
Single NE	15
Combined (ND&NE)	16
Control	0

Table (3): Protective efficacy of the prepared vaccines against challenge with virulent ND virus

Used vaccine	Number of vaccinated chickens	Number of challenged chickens	Number of survived chickens	Protection Percentage %
Single ND	25	15	13	87
Combined (ND&NE)	25	15 against ND	14	93
Control	25	25 against ND	0	0

Usually vaccinologists search to provide potent vaccines to safe poultry and animal wealth in a manner that safe time; cost and stress factors on animal and birds due to application of different vaccines. During the present work a combined oil vaccine was successfully prepared against ND and NE which was found to be stable; free from foreign contaminants ; (aerobic and anaerobic bacteria; fungi and mycoplasma); safe in vaccinated birds where such birds remained healthy allover the experimental period without local reaction at the site of inoculation and immunogenic. These observations agree with the recommendations of USA-CER (1987).

Table (1) showed that vaccinated chickens with single or combined ND and NE vaccines exhibited good levels of specific ND antibodies as estimated by HI test over the recommended protective titer (2^6). These antibodies recorded their peaks (8 &7log2) by the 6^{th} and 7^{th} week post vaccination with the combined ND and NE and single ND vaccines respectively. These results come in agreement with what reported by Chen *et al* (1993) who concluded that formalin inactivated oil emulsion ND vaccine induces protection persisted for more than 6 months. Such findings showed that there is no antagonizing effect between ND and NE antigens on the immune response of chickens against each other. The detected ND antibodies in vaccinated chickens were within the protective levels where they showed protection percentages of 87% and 93% respectively while unvaccinated challenged chickens did not withstand the virulent virus (table-3) with single and combined ND and NE vaccines respectively against virulent ND virus confirmed by the findings of Abo-Zaid *et al* (2001); and Hanan *et al* (2006) who obtained similar results with poultry vaccination with inactivated ND vaccine either in single form or in combination with other viral or bacterial vaccines indicating that inactivated ND virus did not antagonize the immune response of vaccinated birds to other vaccine antigens.

Regarding the immune response of vaccinated chickens to NE in single and combined form with ND vaccines, table (2) showed that all vaccinated birds exhibited good levels of specific NE antibodies over the

recommended protective titer (0.5 IU/ml) as shown by mice serum neutralization test (15 and 16 IU) by 2 weeks post the second dose of the single and combined NE and ND vaccines respectively. The successfulness of NE vaccine to protect chickens was recorded by Jacobs *et al* (1992); Atle *et al* (2004); El-Meneisy *et al* (2007) and El-Sehemy *et al* (2008) who stated that NE vaccine was able to induce high immune response with lower mortality rate with increase in body weight gain in vaccinated chickens than in unvaccinated chickens.

So, it could be concluded that the combined ND and NE vaccines is of good quality and able to protect chickens against the two diseases.

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تحضير وتقييم لقاح ثنائى زيتى مثبط ضد مرضى النيوكاسل والإلتهاب المعوى التنكرزى فى الدجاج

عويس جلال عفان سلمان*، هناء عبد العزيز مصطفى**، غادة محمد صادق**، عبد المنعم سيد حسين*، إلهام عطا الإبيارى**، محمد حسن خضير*، محمد على مخاريطة**

*معهد بحوث الأمصال واللقاحات البيطرية-العباسية- القاهرة
**المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية

يمثل هذا العمل محاولة لتحضير لقاح ثنائى مثبط زيتى ضد كل من مرض النيوكاسل والإلتهاب المعوى التنكرزى ولقد أظهرت نتائج التجارب العملية خلو هذا اللقاح من الملوثات البكتيرية والفطرية والميكوبلازما كما انه ثابت وآمن وفعال حيث لم تظهر الطيور المحصنة أية أعراض مرضية عامة أو موضعية مكان الحقن. هذا وقد اكتسبت الطيور المحصنة مستويات مناعية نوعية عالية لكل من النيوكاسل والإلتهاب المعوى التنكرزى سواء المحصنة باللقاحات الأحادية أو بالقاح المركب المحضر وذلك من خلال نتائج اختبارى مانع التلزن المعوى للنيوكاسل والمصل المتعادل فى الفئران للإلتهاب المعوى التنكرزى. وقد اظهرت الطيور المحصنة نسب حماية ضد التحدى بفيروس النيوكاسل الضارى بلغت87 % ، 93 % للقاح النيوكاسل الأحادى والمركب على التوالى مما يلاحظ معه أن اللقاح المركب يعطى نتائج افضل قليلا من الأحادى الأمر الذى يمكن القول معه بأن اللقاح المحضر كافى لتوفير الحماية المطلوبة للدجاج ضد كل من النيوكاسل والإلتهاب المعوى التنكرزى.