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IMMUNOLOGICAL STUDY ON CHICKENS VACCINATED WITH A COMBINED INACTIVATED SALMONELLA ENTERITIDIS AND NEWCASTLE DISEASE VIRUS (ND) VACCINE

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ABSTRACT

Immunogenicity of a locally prepared combined inactivated oil emulsified Salmonella enteritidis and Newcastle disease virus(SE-ND) vaccine was evaluated in 3-weeks old chickens. The immune response against single Salmonella enteritidis, Newcastle disease and combined (SE-ND) vaccines as measured by ELISA and heamagglutination inhibition (HI) test revealed no substantial differences with respect to the protective values between single and combined vaccine.The results of challenge test showed that vaccinated chickens were effectively protected against virulent strains of Salmonella enteritidis and Newcastle disease virus. The obtained results confirmed that the locally prepared combined inactivated Salmonella enteritidis and Newcastle disease virus was safe, potent and can effectively protect chickens against Salmonella enteritidis and Newcastle disease virus infection.

INTRODUCTION

Among the bacterial and viral diseases of domestic poultry ,infection with Salmonella enteritidis and Newcastle disease are of primary importance to poultry industry. Both diseases continue to be a major cause of high mortality rate in many poultry farms ***(Sinkovics and Horvath, 2000 and Scanes, 2007).***

Salmonellosis is considered to be one of the important causative agent which infect poultry farms specially that which apply the modern intensive system of rearing and management ***(Nakamura et al,1994).***

Control of Salmonellosis in poultry is posing itself as one of the difficult problems not only for those who are concerned with poultry industry ,but also for public health hazard because of the fact that most of the serovars of Salmonella which poultry harbour can act as potential pathogens for man ,this problem has indicated an increasing requirement for effective vaccines to control this important zoonotic infection ***(Methner et al, 2006 and Barrow, 2007).***

On other side,Newcastle disease (ND)is one of the oldest infectious diseases affecting many domestic and wild avian species ,but it is the most notable in domestic poultry due to their high susceptibility and potential for sever impacts of an endemic on poultry industries .The causative agent , Newcastle disease virus(NDV) clinical signs are extremely variable depending on the strain of virus ,species ,age of bird concurrent disease and pre-existing immunity. NDV is so virulent that many birds die without showing any clinical signs .A death rate of almost 100% can occure in unvaccinated poultry flocks ***(Alexander, 2000).***

The disease control depends mainly on administration of a specific potent vaccines by the suitable route at the suitable age ***(Westbury, 1984).***

The present work aim to provide a double protection for chickens against two of the dangerous diseses,Salmonellosis and Newcastle disease,affecting them in a dramatic form saving time and efforts.

MATERIALS AND METHODS

1- Salmonella enteritidis:

A local isolate of Salmonella enteritidis isolated from chickens was obtained from Vet. serum and Vaccine Research institute,Abbasia ,Cairo,Egypt. This isolate was used for experimental vaccines preparation and challenge of vaccinated chickens.

2- Newcastle disease virus (NDV):

2.1. Lasota virus strain:

It was supplied by the Central Veterinary Laboratory, Weighbridge, England, with a titer of 10^{11} EID_{50} /ml .It was used for preparation of experimental single and combined vaccines. NDV was propagated in specific pathogen free (SPF) embryonated chicken eggs according to ***Allan et al (1973).***

2.2. Velogenic viscerotropic NDV:

It was used for challenge test and it had a titer of 10^{6} EID_{50} .It was obtained from Newcastle Disease Research Dept., Vet. Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo.

3- Chickens:

Two hundered of 3-week- old specific pathogen free (SPF) chickens were supplied by the Central Laboratory for Quality Control of Veterinary Biologics ,Abbasia,Cairo These birds were divided as follow:

* 75 chickens were used in the safety test of the prepared vaccines.

* 125 chickens were used in the potency test.

4- Mice:

A total of 250 Swiss albino mice of about 15-20 g body weight supplied by Veterinary Serum and Vaccine Research Institute, were used for determination of LD_{50} of Salmonella enteritidis

5- Vaccine preparation:

5.1. Single Salmonella enteritidis(SE) vaccine:

Inactivated double oil emulsified Salmonella enteritidis vaccine was prepared according to ***Timms et al(1990).***

The final bacterial suspension was adjusted to contain 10^{10} colony forming units/ml then inactivated by adding 0.3% formalin.

5.2. Single Newcastle disease virus vaccine(NDV):

Inactivated double emulsified ND vaccine was prepared according to ***Allan et al (1973).***

5.3. Combined Salmonella enteritidis and Newcastle disease vaccine (SE-ND):

It was prepared according to ***stone et al (1978)*** by mixing previously prepared inactivated SE and ND vaccines by equal volumes ,then adjuvanted with white mineral oil using double emulsification method (water in oil in water).

6- Quality control testing of the prepared experimental vaccines:

6.1- Emulsion stability:

Drop test was carried out according to ***Becher (1965)*** where 2 drops of the emulsion were placed separately on a clean glass slide and each drop was mixed well either with a drop of oil or water. Water in oil emulsion blends readily with oil but does not with water. In addition, vaccine storage at 4°c did not result in separation of the oil from the aqueous phase.

6.2- Sterility test:

Testing the freedom of the prepared vaccines from foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma) was carried out following the directions of the ***OIE(2008).***

6.3- Safety test:

Safety of the prepared vaccines were tested according to ***OIE (2008)*** through inoculation of double dose subcutaneously in each of 25 SPF chickens which kept under daily observation for 14 days.

6.4- Potency test:

Single SE as well as single ND vaccines each was inoculated in a group of 25 SPF chickens and 50 SPF chickens were used for the combined SE-ND vaccine. All vaccines were inoculated through the subcutaneous route with a dose of 0.5 ml administrated twice with 3 weeks intervals. In addition a group of 25 chickens were kept without vaccination as control.

All birds were housed in separate isolates under hygienic measures receiving adequate ration and water. Serum samples were obtained regularly on week intervals to follow up the induced antibody levels up to 10 weeks post the first vaccination.

7- Serological evaluation of humoral immune response:

7.1. Enzyme linked immunosorbent assay(ELISA):

It was carried out according to ***Vollar et al (1976)*** for the determination of Salmonella enteritidis(SE) antibody titers in chicken sera.

7.2. Haemagglutination inhibition (HI) test:

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

8- Challenge test:

8.1. Challenge with virulent Salmonella enteritidis(SE):

Vaccinated chickens with SE vaccines (single and combined) and non-vaccinated chickens were challenged with the virulent Salmonella enteritidis using 0.1ml of 10^8 colony forming units/ml inoculated intramuscularly according to ***Adriaesen et al (2007).***

8.2. Challenge with virulent ND virus:

Vaccinated chickens with ND vaccines (single and combined) and non- vaccinated chickens were challenged with velogenic viscerotropic ND virus using 0.25 ml/bird containing 10^6 EID_{50} /ml via intramuscular route according to ***Reda and Shebble (1976).***

9- Shedding of Salmonella enteritidis(SE) from challenged chickens:

Shedding of Salmonella enteritidis was detected in fecal samples collected from challenged vaccinated and non-vaccinated chickens weekly up to 4 weeks post challenge using Salmonella Shegella medium.

10- Recovery of Salmonella enteritidis(SE) from challenged chickens

On the fourth week post challenge, samples were collected from the heart blood, liver, spleen and caecal junction from vaccinated and non-vaccinated challenged chickens for recovery of the organism.

RESULTS AND DISCUSSION

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 Salmonella enteritidis and Newcastle disease virus (SE-ND) which found to be stable ,free from foreign contaminant (aerobic and anaerobic bacteria ,fungi and mycoplasma) and safe in vaccinated birds where such birds remained healthy all over the experimental period with slight local reaction at the site of inoculation .These observations agree with the recommmendation of ***USA-CER(2007).***

Table (1) showed the antibody titers of ELISA that was carried out on serum samples obtained from vaccinated chickens with single SE or combined SE-ND vaccines exhibited a good levels of specific antibodies against SE by the 1st week(898&906) and reached to the maximum value (3815&3800) on the 10th week post vaccination with single SE and combined SE-ND vaccines respectively.

Concerning the protection efficacy of the prepared SE vaccines either single or combined with ND vaccine, Table (2) showed that the protection rate were 80% and 84% in chickens vaccinated with single SE and combined SE-ND vaccines respectively, while the control unvaccinated group were unable to withstand the experimental infection confirming that the prepared vaccines were effectively potent and hence able to protect chickens against infection. These results came in agree with that reported by ***Uyttbroek et al (1989), Nakamura et al (1994) and barrow (2007)*** who recommended the use of formalized inactivated oil emulsion S.enteritidis vaccine for protection of chickens against infection.

The results demonstrated in Table (3) revealed that S. enteritidis could be detected in fecal shedding of challenged vaccinated birds at ratio of 26.7% and 20% on the 1st week post challenge from birds vaccinated with single SE and Combined SE-ND vaccines respectively and it could not be detected completely by the 4th week post challenge ,while these ratio were 80% and 25% from challenged control birds at 1st week and 4th week post challenge, respectively.

On other hand ,Table (4)showed that the Salmonella organism could be re-isolated from vaccinated challenged chickens either with single SE or combined SE-ND vaccine with ratio 20 to 30% from heart blood, liver, spleen and caecal junction on the 4th week post challenge ,while these ratio were 50 to 75% from control unvaccinated birds.

These results agreed with ***Uyttbroek et al (1989) and Timms et al (1990)*** who found that Salmonella vaccine protects against experimental challenge with shedding of the organism on the same period with

declined rate post challenge with indication that the highest incidence of the organism is that in the caecal junction.

Regarding the immune response of the vaccinated chickens to single ND and combined SE-ND vaccines exhibited good levels of specific ND antibodies estimated by HI test.

These antibodies recorded their peak (10 $\log_2$) by the 8^{th} and 9^{th} week post vaccination with single ND and combined SE-ND vaccines respectively. Such results come in agreement with that reported ***by Chen et al(1993)*** who concluded that formalin inactivated oil emulsion ND vaccine induces protection persisted for more than 6 months. Such finding showed that there was no antagonizing effect between ND and SE 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 percentage of 100% ,while unvaccinated control chickens did not withstand the virulent virus as shown in Table (6) with both single ND and combined SE-ND vaccines against virulent ND virus confirmed by the findings of ***Abo-Zaid et al(2000) 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 the vaccine antigens.

So, it could be concluded that the combined experimentally Salmonella and Newcastle disease vaccine was of good quality and able to protect chickens effectively against both diseases.

Table (1): Salmonella enteritidis (SE)antibody titers in chickens sera measured by ELISA.

Type of vaccine	Salmonella enteritidis antibody titers/weeks post vaccination										
	0	1	2	3	4	5	6	7	8	9	10
Single SE Vaccine	232	898	1468	1680	2533	2772	3011	3224	3478	3716	3815
Combined SE-ND vaccine	212	906	1451	1723	2486	2699	2997	3147	3412	3698	3800
Control	210	2011	233	241	228	225	234	221	228	221	237

Table (2): Protective efficacy of the prepared vaccines against challenge with virulent Salmonella enteritidis.

Type of vaccine	No of challenged chickens	No of survived chickens	Protection %
Single SE vaccine	25	20	80%
Combined SE-ND vaccine	25	21	84%
Control	15	4	26.7%

Table (3): Fecal shedding of Salmonella enteritidis (SE) from challenged chickens.

Type of vaccine	No of positive shedding/ survived birds on weeks post challenge			
	1WPC	2WPC	3WPC	4WPV
Single SE Vaccine	4/15 (26.7%)	3/15 (20%)	1/15 (6.6%)	0/15 (0%)
Combined SE-ND vaccine	3/15(20%)	2/15(13.3%)	0/15 (0%)	0/15 (0%)
Control	8/10(80%)	5/7(71.4%)	2/5(40%)	1/ 4(25%)

*WPC= week post challenge

Table (4): Recovery of Salmonella enteritidis (SE) from challenged chickens.

Type of vaccine	No of positive samples for Salmonella enteritidis recovery			
	Heart blood	Liver	Spleen	Caecal junction
Single SE vaccine	5/20(25%)	4/20(20%)	4/20(20%)	6/20(30%)
Combined SE-ND vaccine	4/20(20%)	4/20(20%)	5/20(25%)	6/20(30%)
Control	3/4(75%)	3/4(75%)	2/4(50%)	3/4(75%)

Table (5): Mean ND haemagglutination inhibition (HI) antibody titers in chicken sera.

Type of vaccine	ND-HI antibody titers(log2/ml) /weeks post vaccination										
	0	1	2	3	4	5	6	7	8	9	10
Single ND Vaccine	0	2	4	5	6	7	8	9	10	10	10
Combined SE-ND vaccine	0	2	3	4	5	6	7	8.5	9	10	10
Control	0	0	0	0	0	0	0	0	0	0	0

Table (6): Protective efficacy of the prepared vaccines against challenge with virulent with ND virus.

Type of vaccine	No of challenged chickens	No of survived chickens	Protection %
Single ND vaccine	25	25	100%
Combined SE-ND vaccine	25	25	100%
Control	10	0	0%

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دراسة مناعية للدجاج المحصن بلقاح مركب ميت ضد مرضى السالمونيلا و النيوكاسل

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تم خلال هذا العمل تحضير لقاح مركب مثبط زيتى مزدوج الاستحلاب ضد كل من عدوى السالمونيلا انتريتيدس و النيوكاسل حيث تبين أن هذا اللقاح امن وفعال لم يحدث ايه ردود فعل غير مرغوبه بعد التحصين ونتج عنه معلبير عالية من الأجسام المناعية لكلا المرضين فى نفس المستويات الذي احدثته اللقاحات الأحادية كما ثبت ذلك من اختباري الاليزا واختبار مانع التلزن الدموى مما يعنى ان خلط الميكروبين فى لقاح واحد لم يؤثر على الاستجابة المناعية لكلا المرضين. كما أثبتت تجارب تحدى المناعة في الدجاج المحصن ان اللقاح المركب يعمل بكفاءة دون التأثير بخلط الميكروبين معا.

مما سبق يتضح أن اللقاح المركب هو لقاح آمن وفعال ذو قدرة على وقاية الدجاج ضد عدوى السالمونيلا انتريتيدس ومرض النيوكاسل.