Immunological studies on a modified adjuvanted fowl cho vaccine

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Abstract

Montanide ISA 70 oil was used in this study as an adjuvant in a compariso the whiterex 309 white oil with Span 80 for preparation of an inactivate cholera vaccine. Sixty chickens were divided into four groups where gro was vaccinated with fowl cholera vaccine containing white oil with spa group (2) was vaccinated with fowl cholera vaccine containing montanide I at ratio 50/50 oil, group (3) was vaccinated with fowl cholera vaccine Montanide ISA 70 at ratio 70/30 while, group (4) was kept as non-vacc control. Inflammatory reactions induced by fowl cholera vaccine containin 70 were mild and transient as compared with those induced by vaccin white oil. Indirect haemagglutination test revealed that all types of the pre vaccines induced high and protective antibody titres. Fowl cholera vaccin Montanide ISA 70 at ratio 50/50 induced earlier and prolonged response the other two vaccines. These results were confirmed by the challeng against virulent fowl cholera strains where the highest protection level (were induced by the last mentioned vaccine.

Introduction

Fowl cholera is considered one of the most important bacterial disease affect poultry industry in Egypt (Gergis, 1987). It is a contagious septic disease associated with high morbidity and high mortality (Saif, 2008).utl of this disease have great economic importance for poultry industry due rapidly of spread and the extra-ordinary virulence shown by many (Rhoads and Rimiler, 1990 and OIE Manual, 1990).Vaccination is the important and economic mean for controlling the disease and has contribu the eradication of other infectious diseases (Hussain, 1994). The ne continuous improvement of the traditional fowl cholera vaccines shou seriously considered.

Oil adjuvant vaccine formulated as water in oil emulsion (w/o) with liquid p have been used for enhancement of immune activity by the slow rele solutes from the aqueous phase (Bennejean et al., 1978, Box and Ellis, Stone, 1991 and Peleg et al., 1993). The major drawback of using w/o em vaccine is their undesirable local side effects such as granulomas and which were sometimes occur at the site of injection (Reid and Blackall, Mineral oil emulsions have been used with antigens to potentiate and p the antibody response (Cox and Coulter, 1997). Several authors as Barne (1996, 1998); Abd El-Hady et al. (2002) and Kamal et al. (2004) used se oil adjuvant of Montanide (Seppic, France) as Montanide ISA 25, 206, achieved better improvement of the immune response post vaccination new oil formulations are of low viscosity, lower reactivity and high poten present work was designed to use the new formulation of Montanide ISA two different ratios in comparison with the white oil (paraffin) in a trial to ir

the efficacy of fowl cholera vaccine by potentiating the protective antibody response in chicken for prevention of fowl cholera disease.

Material and Methods

1. Chicken:

Sixty chickens, six weeks old, were used in this study. They were obtained from commercial poultry farm. They were apparently healthy and had neither history of fowl cholera disease nor previous immunization with fowl cholera vaccine.

2. Strains:

Pasteurella multocida serovars (A and D) were used for preparation of the experimental vaccine and they obtained from Aerobic Bacterial Vaccines Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. These strains were most commonly encountered among poultry infected with fowl cholera in Egypt. Capsular antigen was prepared for measuring the levels of antibodies in sera of chickens according to Carter and Rappy (1962).

3. Adjuvants:

Two adjuvants were used in preparation of the vaccines used in the present study.

a. Montanide ISA 70:

It is a mineral oil based adjuvant from complex water in oil emulsion and mixed with the vaccine according to the manufacturer's instructions. It was obtained from SEPPIC, Paris, France, Batch No. T81461. It was used by two ratios 70/30 and 50/50.

b. Whiterex 309, white oil quality, FDA/A/USP with span 80 as a classical emulsifier:

- Paraffin oil MICBIL, Alexandria.
- Span 80 supplied by Ubichem LTD.
- Tween 80 supplied by Sigma Co., USA.

4. Preparation of vaccines:

a. Preparation of inactivated Pasteurella multocida vaccine:

The avirulent local strains of P. multocida A and D were propagated in trypticase soya broth at 37°C aerobically for 24 hours to obtain a dense culture containing approximately 3.25 x 10^{10} colony forming units (CFU) of each strain/ml (Kucera et al., 1981). After inactivation by addition of 0.5 % formalin for 18-24 hours, each culture was tested for purity, safety and sterility as mentioned by Mukkur et al. (1982). Finally, cultures were equally mixed together then preserved with 0.01 % of thiomersal and stored at 4°C until used.

Three forms of the vaccine were formulated as follow:

a. Oil adjuvant vaccine:

It was prepared according to Stone et al. (1978). The bacteria suspension was mixed to the tween 80 making aqueous phase. Oil phase consisted of paraffin oil and Span. The aqueous phase was emulsified in oil phase by ratio of 1:1.

b. The water in oil vaccine (Montanide ISA 70, Sepic, France:

It was prepared by adding:

1. Equal parts of antigen and oil phase, by ratio of 50/50.
2. One volume of antigen to 3 volume of oil approximately to a ratio of 70/30.

5. Characterization of the vaccine:

For evaluation of the emulsification process of vaccines drop t emulsion viscosity and emulsion stability were done according to Geneidy e (1971), Becher (1965) and Cruickshank et al. (1975) before being used in vaccination program.

6. Standardization of vaccine formulations:

The aforementioned vaccine formulation were subjected to a numbe quality control tests based on sterility, safety criteria following Code of Ameri Federal Regulation (1985) before being used in the vaccination program.

7. Experimental design:

Chickens were divided into 4 groups each of fifteen.

* Random blood samples were aseptically collected from each group of chic just before vaccination.

Group (1): Vaccinated subcutaneously at the middle part of the neck with 0.5 of avian cholera vaccine with mineral oil and Span 80.

Group (2): Vaccinated subcutaneously at the middle part of the neck with 0.5 of avian cholera vaccine with oil Montanide ISA 70 in ratio 50/50.

Group (3): Vaccinated subcutaneously at the middle part of the neck with 0.5 of avian cholera vaccine with oil Montanide ISA 70 in ratio 70/30.

Group (4): Kept as non-vaccinated control group.

Booster vaccination was conducted 4 weeks after the prim vaccination.

8. Serological testing:

Blood samples were aseptically collected from chickens just bef vaccination then after 7, 15, 30, 45, 60, 75, 90, 120 and 150 days p vaccination.

Sera were separated and stored at -20°C until tested for estimatio humoral immune response to fowl cholera vaccines by use of pas haemagglutination test.

Passive haemagglutination test:

Antibodies were measured by the passive haemagglutination test described by **Carter and Rappy (1962)** in microtitre plates by using formali RBCs and capsular antigen of P. Multocida A and D. Performance of the tes two fold dilution of the sera. The vaccine is concluded effective if it ind seroconversion in sera of vaccinated chickens.

9. Challenge test:

Ten chickens from each group were exposed to challenge aga virulent strains of P. multocida serotypes A, D which used in preparation of vaccine. Cell suspension was prepared in concentration 10^9 CFU/ml underg serial dilution, 0.1 ml of virulent P. multocida cell suspensions containing LD_{50} were injected S/C in all chickens three weeks post the secon vaccination. All chickens were observed for 10 days and mortalities w recorded.

Results and Discussion

Fowl cholera is peracute, acute or chronic septicaemic disease for chickens turkeys, it cause high mortality specially in the acute phase (Saif, 2008). F cholera vaccination programs have been developed as prophylactic me against the disease (Azzam et al., 1992). The present study was aimin prepare a modified fowl cholera vaccine using Montanide ISA 70 as a

adjuvant which is a new generation of adjuvant produced by Seppic, France and able to enhace local and systemic immune response, in comparing with the other classical vaccine which contain whiterex 309 white oil with Span 80. The advantage of the newer adjuvant ISA 70 is that it can be used at different ratios 70/30 and 50/50, it has its own emulsifier, and easily prepared. No local reactions are observed, no pyrogenic properties induced by such vaccines as also reported by Barnett et al. (2002) and Yamanaka et al. (1993).

In the present study, serological assay for P. multocida antibodies as represented by indirect haemagglutination test are shown in table (1) and Fig (1). The three formulations of the vaccine gave significant levels of antibodies in vaccinated birds in comparing to the non-vaccinated ones. During the first two weeks post primary vaccination, the antibody titres were elevated, there were slight differences between the three forms, the Montaide ISA 70. oil adjuvant when used by ratio of 50/50 gave early and higher humoral immune response more than the classic oil adjuvant and the other formulation of ISA 70 at ratio 70/30, where the antibody titre reached the peak at 4 weeks post vaccination and persisted at high level for up to 16 weeks.

These findings were in agreement with that of Abdel Hady et al. (2002) who used ISA 70 as an adjuvant for Newcastle vaccine in chicken, and Barnett et al (1996) who used Montanide oil and found that it is the most potent adjuvant for FMD antigen in addition to its superior stability performance. Table (2) described the results of challenge test of vaccinated chickens 100% protection was recorded in chickens vaccinated with the avian cholera vaccine contained Montanide oil ISA 70 in ratio of 50/50 while chicken received the same vaccine with 70% ratio showed 80% protection and chicken which received the avian cholera vaccine contained Whiterex 309 white oil with Span 80 showed 90% protection.

Table (1): P. multocida antibodies in sera of chickens vaccinated with fowl cholera vaccine formulations as measured by indirect haemagglutination test

Type of vaccine	Prevacc.	Weeks post vaccination										
		1 w	2 w	4 w		6 w	8 w	10 w	12 w	14 w	16 w	20 w
1. Avian cholera vaccine using mineral oil	2	64	128	197	2nd dose of vaccination	256	512	512	470	256	226	197
2. Avian cholera vaccine using ISA 70, 50/50	2	64	184	256		256	512	470	226	190	170	128
3. Avian cholera vaccine using ISA 70, 70/30	2	32	64	170		190	256	512	256	256	128	64
4. Non-vaccinated control	2	4	2	6		2	6	8	4	2	4	8

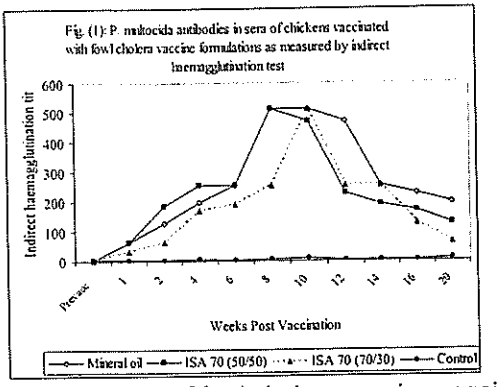

Table (2): Comparative efficiency of fowl cholera vaccines against challenge w
P. multocida serotype A and D

Chicken group and type of vaccine	No. of challenged chicken	No. of dead chicken	No. of survival chicken	Protection rate %
1. Avian cholera vaccine using mineral oil	10	1	9	90 %
2. Avian cholera vaccine using ISA 70, 50/50	10	0	10	100 %
3. Avian cholera vaccine using ISA 70, 70/30	10	2	8	80 %
4. Non-vaccinated control	10	10	0	0

* Challenge was conducted twenty one days after second vaccination.

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لخص العربي

سات مناعية على لقاح كوليرا الطيور المطور

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هذه الدراسة تم استخدام المونتانيد (ISA 70) كمساعد مناعى بتركيزين (٣٠/٧٠)، (٥٠/٥٠) وكذلك ايضاً البارافين وتم تقسيم ستون دجاجة الى ثلاث مجموعات محصنة كالتالى: مجموعة (١) محصنة بلقاح كوليرا ور باستخدام زيت البارافين، مجموعة (٢) محصنة بلقاح كوليرا الطيور بالمونتانيد (٥٠/٥٠)، مجموعة (٣) سنة بلقاح كوليرا الطيور باستخدام المونتانيد (٣٠/٧٠) ومجموعة (٤) غير محصنة. اللقاح مضاف اليه تانيد ISA 70 كان اسهل عند الحقن وأحدث التهابات جلدية بسيطة ومؤقتة بعد الحقن. أظهرت نتائج اختبار ن الدموى الغير مباشر فى المجموعة الأولى التى استخدم فيها زيت البارافين مستوى مناعى عالى ولكن ر عن المجموعة الثانية والتى استخدم فيها المونتانيد بنسبة (٥٠/٥٠) حيث ارتفع المستوى المناعى مبكرا ا عن المجموعة الثالثة والتى استخدمت فيها نسبة (٣٠/٧٠) ولقد تأكدت هذه النتائج باختبار التحدى ضد كوليرا ور والذى أظهر أعلى نسبة حماية (١٠٠%) فى المجموعة رقم (٢) المحصنة بلقاح كوليرا الطيور المحتوى المونتانيد بنسبة (٥٠/٥٠).