Trials For Preparation Of A Combined Vaccine Against *Salmonella Enteritidis* And Egg Drop Syndrome Disease In Chicken

Elham A Youssef , Eman A Hassan and Amina A Elbayomy
Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

ABSTRACT

One hundred and twenty SPF chickens were divided into four equal groups (30 birds for each). The first and second groups were vaccinated with inactivated *Salmonella Enteritidis* (S.E) and Egg drop syndrome (EDS) inactivated oil adjuvant vaccine respectively. The third group was vaccinated with combined S.E and EDS inactivated oil adjuvant vaccine while the last group was kept as non vaccinated control. Each group received first dose of vaccine at 4 weeks old followed by second dose 4 weeks later from the first dose and boostered at 16 weeks before the onset of egg production .Blood samples were collected weekly for determination of humoral immune response, which estimated by using microagglutination test (MAT) and Enzyme Linked Immunosorbent Assay (ELISA) for S .Enteridis ,direct haemagglutination inhibition (HIT) and ELISA test for EDS vaccine .Higher antibody response was observed in vaccinated groups at Third, seventh & Twentieth weeks after first vaccination .

The results of the a previous test proved that the monovalent and combined vaccine induced good protection against both diseases. The potency test ,fecal shedding and reisolation from the internal organs after challenge supported these results, also there was no interference between bacterial and viral inactivated antigens on the immune stimulant of vaccinated fowls to each other. In conclusion the bosstered dose of vaccine before the onset of egg production gave good results in the immune response against both diseases.

INTRODUCTION

Salmonella Enteridis and Egg drop syndrome are two important diseases affecting poultry. Salmonella remains the main food borne bacterial diseases affecting human *(1)*, It is the major cause of food born disease in human over the last 20 years, during which contaminated egg were the most important vehicle of the infection *(2)*. Salmonella are introduced in poultry farms by several ways , including day old iufected chick , domestic animals, humans , equipment, water , food ,the presence of wild birds, rodents, and insects *(1)*. Infected breeder flocks are responsible for vertical transmission , the eggs are contaminated either from the ovary or during the passage through the cloacal feces from infected or carrier hens,the birds survive from clinical disease when infected in young stage may show few signs of infection but they become carriers with or without presence of clinical science and pathological lesions, *(3)* . Birds free of bacteria in contact with inoculated birds become infected and excreted *S.Enteritidis* in the feces following 12 to 24 hours *(4)*.

Laying hens experimentally exposed to *S.enteridis* soon after hatching may remain infected until maturity, producing contaminated eggs and eliminating the bacteria to the environment *(5)*. Once the farms is contaminated, it is difficult to eliminate Salmonella *(6)*.The vaccine is the best method for controlling the disease *(7)*.

The egg drop syndrome (EDS) was originally designated as the solemember of the subgroup III avian adenoviruses but it has been now moved to new genous atodenovirus *(8)*.

Egg drop syndrome is a disease of laying hens characterized by a sudden and frequently large drop in egg production ,with the laying of soft shelled eggs *(9)*. The first sign of Egg drop syndrome is loss of colour of pigmented eggs, quickly followed by production of thin , soft or less shelled eggs *(10)*. The thin shelled eggs often have a rough , sand paper like texture or granular

roughening of the shell at one end . The fall in the production can be very rapid or extended over weeks. Inactivated EDS oil adjuvanted vaccine had produced by Vet. Serum and Vaccine Research Institute (VSVRI) and gave good protection. So the objective of this study was to prepare the bivalent vaccine of *S.Enteridis* and Egg drop syndrome in a single and combined form for protection poultry against diseases caused by these agents before the onset of egg production.

MATERIAL AND METHODS

Strains used

Bacterial strain

S. Enteritidis strain: was kindly obtained from department of Microbiology , Faculty of Veterinary Medicine , Cairo University.

Viral strain

Egg drop syndrome seed virus (EDS) : EDS-76 live virus was supplied by the central Veterinary Laboratory ,Weybridge England with a titre of 10^7 EID_{50}/ ml and haemagglutinating unit was 2^{11} ***(11).***

Laboratory host system

Embryonated chicken eggs

9 to 11 days old embryonated chicken specific pathogen free (SPF) eggs were obtained from Ministry of Agriculture, Koum Osheim, Fayoum, Egypt were used for propagation and titration of the virus EDS76 ***(11).***

Chickens

One hundred and twenty, one day hubberd SPF chicks were obtained from private farm and reared under strict hygienic measures in isolated and disinfected cages till reaching 4 weeks of age.

Cloacal swabs and blood samples were collected from all chickens to be free from *S.enteridis* and EDS virus.

Mice: one hundred Swiss webster mice of 18-20 gm weight were used for passaging of the bacterial strain and safety test of the prepared vaccines.

Adjuvants

It was obtained from Aerobic Bacterial Vaccines Department , Veterinary Serum and Vaccine Research Institute (VSVRI) , Abbasia, Cairo.

Vaccines preparation

S. Enteritidis inactivated oil adjuvant vaccine ***(12).***

Inactivated oil EDS virus vaccine ***(13)*** and combined inactivated oil adjuvant S. E. and EDS vaccine. The combined inactivated oil adjuvant S.E. and EDS was prepared by mixing equal volumes of both inactivated cultures ***(14)*** ,then the prepared vaccine was dispensed into sterile bottle and stored at 4°C till used. The bacterial and virus contents within each dose (0.5ml) were adequately adjusted to be the same in both vaccine.

Quality control of the prepared vaccines

Purity, sterility, safety and potency test were applied to the prepared vaccine according to the recommended procedures ***(15).***

Serological test

Serum sample were collected weekly till 24 weeks and serological tests were carried out by the following tests:

Haemagglutination inhibition test ***(2)*** for EDS virus and Microagglutination test ***(16)*** for *S.enteridis* were carried out. Enzyme linked immunosorbent assay was adopted ***(17)*** for *S. Enteritidis* and ***(18)*** for detection of EDS 76 antibody.

Experimental design :One hundred and twenty of chicken at 4 weeks of age were divided into four groups as shown in Table 1.

Table 1. Experimental design

Vaccinal group	No. of chicken	Dose of vaccine	Time of vaccination	Time of challenge
Group 1: vaccinated with S.E adjuvanted by mineral oil	30	0.5 ml contain $9x10^8$ (mcferland no 1).for S. Entritidis	1st dose at4 week of age.	10 birds challenged at 8 week from 1 st dose of vaccination.
Group2: Vaccinated with EDS adjuvanted by Mineral oil	30	0.5 ml contain 2^{10} Haemagglutinating unit of EDS virus particle	2^{nd} dose after 4 week from the first dose.	10 birds challenged at 20 week post 1^{st} vaccination
Group3: Vaccinated with combined vaccine adjuvanted by mineral oil	30	0.5 ml contain $9x10^8$ for S. Entritidis and 2^{10} EDS virus particle	Booster dose at 16 week from the first dose, before onset of egg production	10birds challenged at 8,20 weeks for the control groups.
Gr4: Control	30	None		Challenge orally with 1 ml contain $3x\ 10^8$ (mcferland no1) for *S. entritidis*

Fecal shedding and detection of S.Enteridis in tissues post challenge: was carried out *(19)*. Groups vaccinated with single or combined and control were challenged with a dose of 1ml contain $3x10^8$ (mcferland No. 1) of *S. Enteritidis* virulent strain . At 8 weeks and 20 weeks from the first dose of vaccine , fecal samples were taken at weekly intervals to observe shedding of *S.Enteridis.* At 12 and 24 weeks in the two groups and control group received challenge were sacrified and internal organs were examined bacteriology.

Protective index was calculated previously *(20)* recorded Table 6.

RESULTS AND DISCUSSION

Salmonella Enteritidis is the more serious disease affecting poultry industries so the vaccination is a practical approach for controlling salmonellosis in chicken.

Egg drop syndrome (EDS) virus causes a drop in egg production and affect the quality of the shell since there is no treatment for EDS infection so the Veterinary Serum and Vaccine research Institute (VSVRI) producing an effective and successfully used inactivated oil adjuvant vaccine .The possibility to produce a combined vaccine against two major diseases affecting layers was tested in this study to reduce and prevent shedding through farms.

The combined vaccines have the advantages of providing protection against more than one disease at the same time thus reducing vaccination expenses and number of vaccination per farm.

Concerning the results of microagglutination and ELISA test observed from the data given in Tables 2,3. That the GMT. of S. Entritidis antibodies was increased to reach the highest value at 3 w, 7 w, 20 w , after 1^{st} vaccination, on the other hand the control non vaccinated group showed steady level at all intervals. Inactivated oil adjuvant *S. Entritidis* vaccine when administered s/c to chicken at 4, 8 weeks has been shown to provide good protection against challenge at 20, 24 weeks *(20)*.

Table 2. Geometric means of antibody titres in sera of chicken vaccinated with different types of vaccine measured by microagglutination test for *S. Entritidis*

Vaccinal Groups	Prevaccination	Weeks post first vaccination																
		1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22	24
Group.1 vaccinated with S.E adju. By mineral oil	0.0	58	113	135	130	145	160	197	193	182	171	155	139	115	190	237	237	237
Group 3 Vaccinated with combined vaccine adju. By mineral oil	0.0	65	130	160	150	156	197	211	208	194	187	164	160	138	208	249	249	249
Group 4 Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 3. Mean absorbance value in sera of chickens vaccinated with different types of vaccine measured by ELISA test for *S. entritidis*

Vaccinal Groups	Prevaccination	Weeks post first vaccination																
		1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22	24
Group1 vaccinated with S.E adju. By mineral oil	0.275	1.65	1.85	2.18	1.90	2.38	2.51	2.78	2.70	2.59	2.45	2.20	1.91	1.60	2.35	2.89	2.89	2.89
Group 3 Vaccinated with combined vaccine adju. By mineral oil	0.273	1.85	2.19	2.30	2.19	2.68	2.70	2.80	2.78	2.71	2.56	2.31	2.25	1.98	2.40	2.99	2.99	2.99
Group 4 Control	0.270	0.279	0.278	0.270	0.261	0.285	0.288	0.279	0.287	0.279	0.281	0.290	0.293	0.295	0.295	0.287	0.293	0.290

EDS virus differ from other Adeno viruses by strongly agglutination of avian RBCs ,as recorded in Table 4 .Geometric mean of antibody titre against EDSV gradually increased at 3weeks post vaccination with a satisfactory titre of 8.5, log2 and 9.4, log2 for groups 2,3 consequently. Testing the effect of the second dose revealed an increasing by 1.5 to 1.7log2 at 7weeks post first dose in the same group, while administered dose before onset of lay induced high antibody titre of (10.3 and 10.8 log2) at 20 w from the first vaccination .The achieved

titre for two groups was acceptable and promising in comparable with that of the recorded protective titre (7log2) as observed by *(12, 21)*.

EDS –ELISA antibody titre Table 5 was in harmony with results of HI, we can notice that ELISA reading were increased at 3,7,20 weeks after 1st vaccination .These results are consistent with previously recorded study *(23)*.

Table 4. Geometric means of haemogglutination inhibition (HIT) antibody titre log2 in sera of vaccinated chickens against EDS virus.

Vaccinal groups	prevaccination	Weeks post 1st vaccination																
		1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22	24
Group2 vaccinated with EDS adju. By mineral oil	0	21	7.0	8.5	8.3	8.5	9.0	10.0	10.0	9.6	9.30	8.8	8.5	8.1	10.1	10.30	10.30	10.28
Group3 vaccinated with combined vaccine adju. By mineral oil	0	2.5	7.6	9.3	8.9	9.2	10.6	10.6	10.9	10.3	9.8	9.4	9.2	8.9	10.7	10.80	10.80	10.79
Group 4 control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5. EDS ELISA antibody absorbance value in groups of chicken vaccinated with different types of vaccines expressed as optical density

Vaccinal groups	prevaccination	Weeks post 1st vaccination																
		1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22	24
Group2 vaccinated with EDS adju. By mineral oil	0.184	1.17	1.33	1.89	1.75	2.15	2.35	2.66	2.60	2.40	2.33	2.16	1.88	1.52	2.74	2.78	2.78	2.76
Group3 vaccinated with combined vaccine adju. By mineral oil	0.183	1.42	1.70	2.15	1.96	2.46	2.63	2.95	2.85	2.63	2.45	2.13	1.80	2.62	3.16	3.15	3.16	3.15
Group 4 control	0.181	0.181	0.185	0.180	0.179	0.170	0.173	0.180	0.189	0.185	0.183	0.183	0.173	0.180	0.179	0.172	0.179	0.180

Negative control values from 0: 0.196
Positive value from 1.05

Results of challenge test against virulent *S. entritidis* was tabulated in Table 6 proved that combined vaccine gave protection percent 80 %, in contrast 60% in the monovalent vaccine at the group received challenge at 8 weeks, while it was 100% in the monovalent and combined vaccine at the groups received challenge at 20 weeks post first vaccination *(20)*.

In comparison with the control group the mortality rate was 50%,40% at challenge at 8,20w respectively. The mortality rate was ranged previously from 35% to 50% *(20,23)*.

Shedding of *S. Entertidis* was lowered in 1st and 2nd weeks at group 1,3 in birds which received challenge at 8 weeks, while its completely eliminated at 3rd, 4 th weeks . The birds at the same groups received challenge at 20 weeks is completely eliminated through the four weeks, in comparison with the control group the shedding was found after challenge till the end of the experiment Table 7 ,this results are similar to that recorded in S.E. adjuvant vaccine *(20)*.

Several factors might be related to the non isolation of *S. entritidis* from fecal samples in groups 1,3 at 3 w, 4 w in the chicken received challenge at 8 w and at 1, 2, 3, 4 w in the group received challenge at 20 weeks due to acquired immunity from vaccine or acquired natural resistance against enteric pathogens with the gradual development of the intestinal flora and the immune system *(24)*.

After challenge the lesions in the birds which were non vaccinated included pericarditis, inflammed ovary and swallen with inflammation caeca, congestion of liver, spleen and enlarged gall bladder. Birds which died within 48 hrs of challenge often manifested by fever and milder lesions than these which died later, in contrast very mild lesion in vaccinated groups received challenge at 8w, no lesion were observed in vaccinated groups which challenged at 20 weeks.

Table 6. Potency Test of chicken vaccinated with *S. entritidis* alone or in a combined form

Groups of chicken	Potency of birds received challenge at 8 w post 1st dose of vaccination							Potency of birds received challenge at 20 w post 1st dose of vaccination						
	N0. of chicken	No. of dead birds / weeks post 1st challenge				Dead/ survived	*Protection percentage	N0. of chicken	No. of dead birds / weeks post 2nd challenge				Dead/ survived	*Protection percentage
		1 w	2 w	3 w	4 w				1 w	2 w	3 w	4 w		
Group 1 Vaccinated with S.E adjuvanted by mineral oil	10	1	1	-	-	2/10	60%	10	-	-	-	-	0/10	100%
Group 3 Vaccinated with combined vaccine adjuvanted by mineral oil	10	1	-	-	-	1/10	80%	10	-	-	-	-	0/10	100%
Group 4 Control	10	3	2	-	-	5/10	-	10	2	2	-	-	4/10	-

* Protection percentage as described *(20)* to calculate protective index (PI) for Salmonella as formula :

$$PI = \frac{\text{Incidence in control} - \text{incidence in vaccinates}}{\text{Incidence in control}} \times 100$$

Table 7. Fecal shedding of *S. enteritidis* after oral challenge of chickens vaccinated with different types of vaccine

Groups of chicken	No of Positive / total number of living birds after challenge at 8 w								No of Positive / total number of living birds after challenge at 20 w							
	1w		2w		3w		4w		1w		2w		3w		4w	
	No of ex. Chicken	No of + ve	No of ex. Chicken	No of + ve	No of ex. Chicken	No of + ve	No of ex. Chicken	No of + ve	No of ex. Chicken	No of + ve	No of ex. Chicken	No of + ve	No of ex. Chicken	No of + ve	No of ex. Chicken	No of + ve
Group 1 vaccinated with S. E vaccine adjuvanted by mineral oil	10	2/10 (20%)	9	1/9 (11.1%)	8	0/8 (0%)	8	0/8 (0%)	10	0/10 (0%)	10	0/10 (0%)	10	0/10 (0%)	10	0/10 (0%)
Group 3 vaccinated with combined vaccine adjuvanted by mineral oil	10	1/10 (10%)	9	1/9 (11.1%)	9	0.9 (0%)	9	0/9 (0%)	10	0/10 (0%)	10	0/10 (0%)	10	0/10 (0%)	10	0/10 (0%)
Group 4 Control	10	6/10 60%	7	3/7 (45%)	5	2/5 (40%)	5	1/5 (20%)	10	5/10 (50%)	8	3/8 (40%)	6	2/6 (33.3%)	6	1/6 (16.6%)

Ex. Examined

As shown in Table 8 the recovery rate of *S. entritidis* from organs in the group received challenge at 8w were 20 % at group 1 vaccinated with monovalent vaccine , but it was 10 % in groups 3 which received a combined *S. Entritidis* and EDS vaccine in comparison with 70 %, in the control group after challenge at 8w .

After challenge at 20 w , the birds given monovalent or bivalent vaccine didn't shed S. entritidis, and the organs were not colonized, in contrast the control groups was 60% recovery from organs. The ability to clear infection in a short period after challenge exposure is an indication of the acquired protection of the antibody response ***(25)*** .

The results obtained from this study indicated that the boostered dose of both vaccines before the onset of egg production gave better results in the immune response. The best vaccination program at 4w and revaccination at 16w of age before starting the season of egg production, gave the highest level of antibodies against EDS ***(26)*** .

From the aforementioned results it could be deduced that combining both S.entritidis and egg drop syndrome antigen had no adverse effect on the immune response of chicken to each of them separately, no mutual interference between the two antigen could be observed when measuring the antibody titres to both of them. So it could be concluded that *S. entritidis* and egg drop syndrome combined prepared vaccine is a safe and potent vaccine, also the booster dose of vaccine before the onset of egg production gave good results against both diseases.

Table 8. Isolation of *S. Entritidis* from the internal organs of chicken vaccinated with different types of vaccines and challenged with Virulent *S. Entritidis*

Groups of chicken	Reisolation at 12 w post 1st vaccination in the group received challenge at 8 w					No of +ve culture / total no of examined chicken	Percent age of + ve culture	Reisolation at 24 w post 1st vaccination in the group received challenge at 20 w					No of +ve culture / total no of examined chicken	Percentage of + ve culture
	Ovary	Oviduct	Spleen	Liver	Caecal junction			Ovary	Oviduct	Spleen	Liver	Caecal junction		
Group 1 Vaccinated with S.E adjuvanted by minera oil	1/10	1/10	1/10	1/10	2/10	2/10	20 %	0/10	0/10	0/10	0/10	0/10	0/10	**0%**
Group 3 Vaccinated with combined vaccine adjuvanted by mineral oil	0/10	0/10	0/10	0/10	0/10	1/10	10%	0/10	0/10	0/10	0/10	0/10	0/10	0%
Group 4 control	2/10	2/10	6/10	6/10	7/10	7/10	70%	2/10	2/10	5/10	5/10	6/10	6/10	60 %

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الملخص العربى

محاولة لتحضير لقاح مركب مثبط ضد مرضى السالمونيلاانتريتيدس وظاهرة تدنى البيض فى الدواجن

الهام علام يوسف ، ايمان احمد حسن على ، امينة احمد البيومى

معهد بحوث الأمصال واللقاحات البيطرية بالعباسية

فى هذه الدراسة تم استخدام 120 دجاجة قسمت الى اربع مجموعات (30 دجاجة فى كل مجموعة). المجموعة الاولى والثانية تم تحصينها باللقاح الاحادى المثبط للسالمونيلا انتريتيدس وظاهرة تدنى البيض كلا على حدة. المجموعة الثالثة تم تحصينها باللقاح المركب المثبط ضد كلا المرضين .تم تحصين كل مجموعة من المجموعات السابقة بجرعة اولى عند عمر 4 اسابيع والجرعة الثانية بعد 4 اسابيع من الاولى ثم حصنت بجرعة منشطة عند 16 اسبوع (من الجرعة الأولي) قبل فترة انتاج البيض .اما المجموعة الرابعة تركت كمجموعة ضابطة . وقد اثبتت التجارب السيرولوجية المختلفة (التلازن البسيط والتلازن الدموى المباشر والاليزا) ان كلا اللقاحين الاحادى والمركب أعطى كفاءة مناعية عالية فى الاسبوع الثالث والسابع والعشرون بعد الجرعة الاولى وقد اكدت هذه النتائج بالعزل من البراز والأعضاء الداخلية بعد اجراء اختبار التحدى.وقد اوضحت النتائج ان خلط ميكروب السالمونيلا انتريتيدس وفيروس تدنى البيض فى لقاح واحد لم يؤثر على الاستجابة المناعية لهذه الإمراض وبناء عليه يوصى باستخدام اللقاح المركب بأمان وكفاءة عالية كما يوصى

استخدام الجرعة المنشطة قبل انتاج البيض لحماية الدجاج من تلك المرضين.