Evaluation Of Immune Response Of Ducks Vaccinated With Live Attenuated Salmonella *Typhimurium Vaccine*

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ABSTRACT

The present work evaluated the immune response of ducks to the live attenuated *Salmonella typhimurium* vaccine where sixty of three weeks old ducklings were vaccinated with such vaccine via drinking water while another forty birds were kept without vaccination as a control. Challenge of these ducklings four weeks post vaccination showed that vaccinated birds were able to withstand the virulent strain of *Salmonella typhimurium* with protection rate reached 90% while non vaccinated birds did not. In addition, the enzyme linked immune sorbent assay revealed that vaccinated ducklings exhibited high specific antibody titers (2915) by the fourth week post vaccination. Shedding of the organism was observed only during the first 2 weeks post vaccination that in cloacal swabs and internal organs. So, it could be concluded that the live attenuated *Salmonella typhimurium* vaccine is able to protect ducklings against the virulent strain.

INTRODUCTION

Salmonella infection is one of the most important bacterial disease affecting poultry industry especially in intensive systems of rearing. Such infection has a public health importance indicating a need to control *Salmonella* infection in poultry *(1)* and any contributions for organism elimination in birds could have a major influence in reduction of its populations under natural conditions *(2)*. As it is well known that vaccination is considered the corner stone in controlling bacterial and viral infections and accordingly control of *Salmonella* infection in ducks is inevitable. Both of live attenuated and inactivated vaccines are available, where live vaccines induce better protection than inactivated ones *(3)* while inactivated vaccines appeal more to producers and regulators because they do not pose the possible public health risk that accompany the use of live vaccines *(4)*.

Live *Salmonella* vaccines replicate; colonize and invade intestinal and visceral organs of inoculated birds, thereby leading to the induction of strong immunity in the vaccinated birds *(5,6)*. However, live vaccines should be avirulent, stable and immunogenic *(7)* and doesn't enhance the development of *Salmonella* carrier status *(8)*.

Ducklings are at high risk in the hatchery and during the first weeks of growth in Salmonella contaminated farms. The development of vaccination program that enhances duck immunity early in life; is an urgent need. So, the present work was aimed to evaluate the immune response of ducks to a live attenuated *Salmonella typhimurium* vaccine in a trial to draw a plan for duck industry protection against Salmonella infection.

MATERIAL AND METHODS

1.*Salmonella typhimurium* vaccine

Live attenuated freeze dried *Salmonella typhimurium* vaccine (Avipro Salmonella Vaccine T) was supplied by Lohman Animal Health Company. On use, the vaccine was reconstituted according to the manufacturer directions where each dose contains 10^8 CFU of the organism.

2. *Salmonella typhimurium* strain

Standard strain of *Salmonella typhimurium* was kindly supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. This strain was used for challenging vaccinated ducks as well as for preparation of the antigen required for ELISA.

3.Experimental hosts

3.1.Ducks

One hundred Muscofy three weeks old ducklings were obtained from El-Wafaa Farm, 6th October Governorate, Egypt. These ducklings were tested and found to be free from Salmonella infection and antibodies as determined serologically. The birds were divided into two groups as follow:

1- Group (1) of 60 ducklings was vaccinated orally with the live attenuated *Salmonella typhimurium* vaccine via drinking water.
2- Group (2) of 40 birds was kept without vaccination as control.

All birds were housed under hygienic measures in separate isolates receiving balanced ration and adequate water.

3.2. Mice

A total of 250 weaned Swiss albino mice of about 25 gm body weights were used for passage and detection of the LD_{50} of Salmonella typhimurium.

4.Challenge test

On the fourth week post vaccination, each duck group was subdivided into two subgroups as follow:

1- Group 1/ subgroup-1, of 30 birds was kept without challenge to follow up the induced immunity in their sera.
2- Group 1/ subgroup-2, was challenged with the virulent strain of *Salmonella typhimurium*.
3- Group 2/ subgroup-1, of 20 birds was kept as control all over the experimental period.
4- Group 2/ subgroup-2, of 20 birds was challenged against the virulent organism.

The challenge was carried out through the intramuscular inoculation with 7×10^8 CFU of the virulent strain /0.1ml ***(9)***. All birds were kept under daily observation for 2 weeks post challenge to record any abnormalities or deaths.

5.Sampling

5.1.Serum samples

Serum samples were obtained from vaccinated ducks in group1/subgroup-1 at weekly intervals for up to four weeks then every month for 3 months post vaccination. The collected samples were used to evaluate the level of induced humeral immunity using ELISA. Serum samples from group 1/ subgroup-2 were obtained at weekly intervals 4 times post vaccination and one time post challenge to follow up the level of detected antibodies.

5.2. Cloaca swabs

Cloaca swabs were obtained from randomly selected 20 vaccinated and 10 control ducks on the 3rd; 7th; 14th and 21st days post vaccination.

5.3. Organ specimens

Randomly selected ducks (10 birds from each group) were scarified twice with one week intervals post vaccination to detect the presences of *Salmonella typhimurium* in specimens of heart, liver, spleen and caecum.

Bacterial determination was carried out through cultivation of prepared samples on *Salmonella Shigella* (SS) agar (Difco)

6.Anti-duck conjugate with horse radish peroxidase

It was supplied by Sigma Company and used in the solid indirect ELISA.

7. *Salmonella typhimurium* antigen

Lipopolysaccharide (LPS) *S.typhimurium* antigen was prepared ***(10)***.

8.Evaluation of the humeral immune response of vaccinated ducks:

The humeral immune response of vaccinated ducks was followed up on regular intervals post vaccination and challenge using the enzyme linked immune sorbent assay (ELISA) ***(11)***.

RESULTS AND DISCUSSION

Salmonella infections in poultry are probably the most important source of *salmonella*-associated food-poisoning in human and the contribution of different species to human infection bears some relationship to the quantity of meat from each species that is consumed. Consumption of duck meat is much greater in some countries where the incidence of human infection

originating from this source *(1)*. Incidence of human infection arising from consumption of duck meat is likely to be much greater as it is with human infection arising from chickens *(12)*. Accordingly, the present study aimed to answer the question about to any extent ducks could be protected against *salmonella* infection? And parallel to this respect how aid to minimize salmonella infection in man?

The present results revealed that vaccination of ducklings with the live attenuated *S. typhimurium* vaccine leads to shedding of the organism from vaccinated birds through the first two weeks post vaccination as determined by culturing of cloacae swabs and internal organs on SS medium (Table 1). *(13, 14) S. typhimurium* from was recorded cloacal swabs obtained from vaccinated chickens during the 14th day post vaccination with the live attenuated vaccine.

Table 2 showed the mean antibody titers of ELISA test that was carried out on serum samples obtained from vaccinated ducklings.It revealed that these birds exhibited high levels of specific antibodies against *S. typhimurium* by the first week (1690) and up to 3 months post vaccination (2915). Table 3 indicated that such titers declined to 2367 one week post challenge with the virulent strain while unvaccinated challenged ducks showed titers of 211-248 pre-challenge and 1213 by the first week post challenge. Similar results with oral vaccination of birds were obtained using ELISA *(8, 15, 14)*. However, it has been suggested that the degree of immune responses to *Salmonella* depend on the host species and the *Salmonella* serotype infection *(16)*.

Table 1. Shedding and recovery of *Salmonella typhimurium* from experimental ducks

Duck groups	Positive recovery from cloaca				Positive recovery from organs		Total positive recovery from organs	% of positive recovery from organs
	3DPV*	7DPV	14DPV	21DPV	7DPV	14DPV		
1	20/20	20/20	16/20	3/20	3/10	5/10	8/20	40%
2	0/10	0/10	0/10	0/10	0/10	0/10	0/20	0%

*DPV= days post vaccination
Goup-1= vaccinated ducks
Group-2= non-vaccinated control ducks

Table 2. Mean antibody titers of indirect solid ELISA applied on the sera of vaccinated ducks

Duck Groups	Mean titers of ELISA on periods post vaccination						
	Pre-V*	1WPV**	2WPV	3WPV	4WPV	2MPV#	3MPV
Group1 Subgroup1	198	1690	2109	2835	2915	2935	2915
Group2 Subgroup1	211	223	234	245	248	234	222

*Pre-V= pre-vaccination **WPV= week post vaccination
#MPV= month post vaccination
Group-1 subgroup-1= vaccinated non-challenged ducks
Group-2 subgroup-1= non-vaccinted non- challenged ducks

Table 3. Mean antibody titers of indirect solid ELISA applied on the sera of vaccinated and challenged ducks

Duck groups	Mean titers of ELISA on weeks post vaccination					First week post challenge
	Pre-vaccination	1 WPV*	2 WPV	3 WPV	4 WPV	
Group1 Subgrou2	198	1690	2109	2835	2915	2367
Group2 Subgroup2	211	223	234	245	248	1213

*WPV= week post vaccination

Group-1 subgroup-2= vaccinated challenged ducks

Group-2 subgroup-2= non-vaccinated challenged control ducks

Challenge of vaccinated ducks did not result in any clinical abnormalities with 40% protection rate, while unvaccinated control birds showed diarrhea and deaths. So the used vaccine could be considered a potent vaccine providing good immune status for vaccinated ducks, where the efficacy of vaccine preparation is judged by the level of intestinal and systemic colonization and morbidity and mortality rates after vaccination and experimental infection using oral or parental routes of administration.

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الملخص العربى

تقييم الاستجابة المناعية للبط المحصن بلقاح السالمونيلا تيفيميوريوم الحى المستضعف

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تتضمنت الدراسة الحالية تقييم استجابة البط المناعية للقاح السالمونيلا تيفيميوريوم الحى المستضعف حيث تم تحصين مجموعة من البط عند عمر ثلاثة أسابيع عن طريق مياه الشرب ثم تم تتبع إفراز الميكروب فى مسحات من المجمع والأعضاء الداخلية حيث ثبت وجود الميكروب وذلك بزرع هذه العينات على الوسط الغذائى الخاص بميكروب السالمونيلا كما أكد قياس المستويات المناعية المتكونة فى أمصال البط المحصن باختبار الأنزيم المرتبط المدمص المناعى أن هذه الطيور تكتسب مناعة جيدة تكفى للتغلب على العدوى بالميكروب الضارى عند إجراء اختبار التحدىبنسبه حمايه تصل الى90% كما أن الطيور المحصنة لم تظهر أية أعراض مرضية سواء بعد التحصين أو التحدى وعلى ذلك يمكن القول بأن لقاح السالمونيلا تيفيميوريوم الحى المستضعف يمكن أن يوفر حماية جيدة للبط ضد العدوى بالميكروب الضارى.