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Estimation of protective indices in chicken vaccinated with single and booster doses of trivalent salmonella vaccine

Abstract

Background: Infection with Salmonella species is a major health concern for human and animals on a global scale. Most cases of Salmonellosis results in complicated diarrhea, elderly and immune-compromised persons can be at risk for more severe invasive infections which can be life threatening. Control of Salmonellosis in poultry by vaccination is a possible means of controlling the problems.

Material and method: Two different inactivated trivalent *S. Enteritidis, S. Typhimurium* and *S. Kentucky* vaccine batches of 2 different origins were used to vaccinate salmonella free chickens with either single dose or single then booster dose vaccination programs. These chickens were reared in clean separated pens and later on were challenged with virulent *S. Enteritidis, S. Typhimurium* and *S. Kentucky* virulent strains 3 weeks post single or booster doses. Then protective indices was estimated as mean of vaccine evaluation.

Results: Vaccinated birds showed varied protection according to challenge strains, vaccination program and origin of vaccine. Protective indices was estimated as 71% and 66.2% for local and commercial inactivated trivalent salmonella vaccine respectively when chicken challenged 3 weeks post single dose vaccination . Protective indices raised up to 82.5% and 79.5% respectively when birds challenged 3 weeks post booster vaccination.

Conclusion: Evaluation of the combined salmonella vaccine depending on protective indices is more obvious and the picture more better than evaluation depending on either measurement of humoral response or mortalities post challenge because of protective indices depends on several parameters reflecting the immune status of the birds including mortalities , clinical signs and post mortem lesions.

Keywords: salmonella vaccine, salmonellosis, *Samonella enteritides, Salmonella typhimurium,*

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Introduction

Salmonellosis is one of the most common food borne bacterial disease in the world, the great majority of salmonella infections in humans are food borne with *Samonella enteritides* and *Salmonella Typhimurium* accounting for a major part of the problem.[1] The genus Salmonella consists of more than 2500 serovares[2] it is reported that approximately 60% of human salmonellosis were caused by *S. typhimurium* well as *Salmonella Enteritidis*.[3]

The global epidemiology of non typhoidal salmonella disease is complex, with diverse serovars in different regions worldwide, bosing a substantial challenge for vaccine development. *Salmonella typhurium* and *Salmonella enteritidis* have been the most prevalent serovars in people and animals for many years. (anglo)Human cases of salmonellosis were found to be caused by *S. shubra, S. enteritidis*, *S. typhimurium* and *S. kentucky* were reported to be wide spread in a study carried out.[4] *Salmonella enterica* is a major global food –born pathogen causing life threatening infection.

Infection with Salmonella species is a major health concern for human and animals on a global scale. Although most cases of Salmonellosis results in complicated diarrhea. Elderly and immune-compromised persons can be at risk for more severe invasive infections which can be life threatening and may be require antimicrobial therapy American pediatric association. Constructed three bivalent vaccines for preventing both *Salmonella typhimurium* and Salmonella new port infections and concluded that the delivery of heterologous antigen is a prospective approach for developing salmonella vaccines.[5]

Control of Salmonella infections 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 the most of serovars of Salmonella harbored by poultry can act as potential pathogens for man.[6] Killed and live attenuated products have been used for controlling salmonella in poultry production and vaccination with live attenuated products has proved to be effective.[7] Prevention of avian salmonellosis using inactivated vaccine has been reported by several authors to provide good protection with decrease or absence of the residual virulence.[8] So, this present work aimed to evaluate the challenge vaccination assay which reflects protection against (*S. enteritidis, S. typhimurium* and) by *S. kentucky* estimating the protective indices.

Material and methods

Vaccine used

Two different batches of combined trivalent inactivated *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella kentucky* were supplied by CLEVB (Central Laboratory for Evaluation of Veterinary Biologics) and used in the evaluation study. The first one represents a local producer (Veterinary Serum and Vaccine Research Institute) while the second one represents a commercial imported vaccine.

Bacterial strain

Local field isolates of *S. enteritis*, *S. typhimurium* and *S. kentucky* were kindly obtained from (CLEVB). These strains were used in challenge processes.

Vaccination –challenge assay

Vaccination program: 570 Salmonella free chickens were reared in clean separated pens and fed on balanced ration. These chickens were divided into 3 groups as following:

a. First group: It consists of 240 chickens and it was subdivided into 2 equal subgroups, first sub group was vaccinated with locally produced trivalent Salmonella vaccine while the second sub group was vaccinated: With the commercial trivalent Salmonella vaccine (120 chicken for each).

b. Second group It comprising of 240 chicken and subdivided into 2 equal subgroups (120 each), first sub group was vaccinated with locally produced trivalent Salmonella vaccine while the second sub group was vaccinated with commercial trivalent salmonella vaccine, after 3weeks, the subgroups were boostered doses from corresponding vaccines.

c. Third group: It was comprising of 90 chicken and was subdivided equally in parallel to other groups kept as control groups.

Vaccination assay: Each group of the two subgroups in the first main group was subdivided into 3 groups each subgroup was challenged intramuscularly with 0.1ml I/M rout of one of *S. enteritidis* 1x10^7CFU/bird, *S. typhimurium* 1x10^6 CFU/bird or *S. kentucky* 1x10^7CFU/bird 3weeks post single dose vaccination in parallel to 15 unvaccinated control chicken for each.

The same treatment was adapted for the vaccinated chicken in the second main group but was challenged 3 weeks post booster dose vaccination.

Calculating the protective indices using the following formula by,[7] protective indices (PIs) were assessed according to mortality and PM lesions (PML)

$$PI = \frac{\%(M\ \&\ PML)controls - \%vaccinated(M\ \&\ PML)}{\%control(M\ \&\ PML)} X100$$

Results

As regarding to the protection obtained in vaccinated chicken after challenge with *Salmonella enteritidis* strain, Table 1 showed that protection obtained Post single dose was (72.5%) while it raised up to (81.25%) 3 weeks post booster vaccination with combined trivalent salmonella vaccine while it was only (13.3%) in the non vaccinated chicken group.

As shown in Table 2 the protective indices achieved with virulent *Salmonella typhimurium* strain challenge after vaccination with trivalent salmonella vaccine was (71.25%) and (81.25%) after single dose and booster dose respectively while it was (6.6%)in the control non vaccinated chicken group.

As regards to protection obtained in the vaccinated chicken after challenge with virulent Salmonella Kentucky strain, Table 3 showed that vaccinated chicken groups with trivalent Salmonella vaccine showed marked protection level more than the control vaccinated chicken. Overall protection after challenging with virulent *Salmonella kentucky* strain were (72.5%) after single dose vaccination which raised up to (81.25%) after booster dose , while it was only (13.3%) in the non vaccinated control group.

As shown in Table 4, protective indices were estimated for challenged chicken post either single dose or booster dose vaccination. Protective indices were 71 and 66.5 for the 2 different local and commercial tested vaccine batches respectively. While it was 82.5 and 79.5 in case of challenge post booster dose of vaccination assay for both tested vaccines respectively.

Table 1 Results of vaccination challenge assay against *Salmonella enteritidis* 3 weeks post single and booster dose vaccination

Booster dose vaccination				Single dose vaccination				
control	Total	Imported	Local	control	Total	Imported	Local	
15	80	40	40	15	80	40	40	No
6	7	4	3	6	10	6	4	M
7	8	4	4	7	12	6	6	S
13	15	8	7	13	22	12	10	T/A
12	15	8	7	12	20	10	10	R
13.30%	81.25%	80%	82.50%	13.30%	72.50%	70%	75%	P %

No, Number of birds; M, Mortalities; S, Symptoms; T/A, Total affected; R, Re isolation; P%, Protection%

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Table 2 Results of vaccination challenge assay against *Salmonella typhimurium* weeks post single and booster dose vaccination

Booster dose vaccination				Single dose vaccination				
control	Total	Imported	Local	control	Total	Imported	Local	
15	80	40	40	15	80	40	40	N
7	7	4	3	7	12	6	6	M
7	9	5	4	7	11	6	5	S
14	15	9	7	14	23	12	11	T/A
12	13	9	7	13	23	12	11	R
6.60%	81.25%	77.50%	82.50%	6.60%	71.25%	70%	72.50%	P %

No, Number of birds; M, Mortalities; S, Symptoms; T/A, Total affected; R, Re isolation; P%, Protection%

Table 3 Results of vaccination challenge assay against Salmonella Kentucky weeks post single and booster dose vaccination

Booster dose vaccination				Single dose vaccination				
Control	Total	Imported	Local	control	Total	Imported	Local	
15	80	40	40	15	80	40	40	N
6	6	3	3	6	9	5	4	M
7	9	5	4	7	13	7	6	S
13	15	8	7	13	22	12	10	T/A
12	14	7	7	13	22	12	10	R
13.3%	**81.25%**	**80%**	**82.5%**	**13.3%**	**72.5%**	**70%**	**75%**	P %

No, Number of birds; M, Mortalities; S, Symptoms; T/A, Total affected; R, Re isolation; P%, Protection%

Table 4 Estimated protective indices post single and booster dose vaccination for local and commercial vaccines

Control	Vaccines										Birds
	Local						Imported				
TA%	SL/T	D/T	PI	TA%	SL/T	D/T	PI	TA%	SL/T	D/T	
13/15	7/15	6/15	71.1	25%	6/40	4/40	65.38	30%	7/40	5/40	SE
14/15	7/15	7/15	70.5	27.5%	5/40	6/40	67.84	30%	6/40	6/40	ST
13/15	7/15	6/15	71.1	25%	6/40	4/40	65.35	30%	7/40	5/40	SK
40/45	**21**	**19**	**71**	**31/120**	**17**	**14**	**66.2**	**36/120**	**20**	**16**	T
13/15	7/15	6/15	79.8	17.5%	4/40	3/40	79.8	17.5%	4/40	3/40	SE
14/15	7/15	7/15	81.2	17.5%	4/40	3/40	75.8	22.5%	5/40	4/40	ST
13/15	7/15	6/15	79.8	17.5%	4/40	3/40	79.8	17.5%	5/40	2/40	SK
40/45	**21**	**19**	**82.5**	**21/120**	**12**	**9**	**79.5**	**23/120**	**14**	**9**	T

Discussion

Inactivated trivalent salmonella vaccine which composed of *S. enteritidis*, *S. typhimurium* and *S. kentucky* now is one of the target vaccines that is used in the control of the most common salmonellae in between poultry and human public health.

As regarding to the protection obtained in the vaccinated chickens after challenge with virulent salmonellae post single or booster doses, evaluation of the protective value of the used trivalent salmonella vaccines using challenge test was used according to.[9] This test is considered the master test for determination of the protective value of a vaccine.[8]

The obtained data showed that the vaccinated chickens gave protection of 72.5% when challenged with virulent *S. enteritidis* strain 3 weeks post single dose vaccination. This protection was raised to 81.25% when birds were challenged 3 weeks post boostering as shown in Table 1. Also chickens showed protection of 71.25% when challenged with virulent *S. typhimurium* strain 3 weeks post single dose vaccination which raised to 81.25% when challenged 3 weeks post boostering as shown in Table 2. Vaccinated Birds gave protection of 72.5% when challenged with virulent S. Kentucky strain 3 weeks post single dose vaccination meanwhile this protection was raised to 81.25% when birds were challenged 3 weeks post boostering as shown in Table 3. The achieved protection values by the 3 vaccine batches are accepted to pass the vaccine for use according to.[10] These

results are nearly in are agree with the results which were obtained by[10–12] immunized chickens with *Salmonella typhimurium* vaccine then subsequently challenged with the virulent strain and concluded that the vaccinated birds showed resistance to infection with virulent *Salmonella typhimurium* and this was reflected in bacterial infection and shedding. Also[13] confirmed that vaccinated birds with killed *Salmonella enteritidis* vaccine can protect them from challenge with virulent organisms at the end of 2nd week post booster dose.[8] Stated that challenge test is the master test for the determination of *Salmonella kentucky* vaccine and[14] reported that the protective value against virulent *Salmonella kentucky* post challenge in chickens vaccinated with *Salmonella kentucky* vaccine was 80% and the achieved protection value by the used vaccine was accepted to pass the vaccine for use according to[15,10].

Concerning the overall protective indices for the vaccinated chickens, it was 71% in case of local vaccine, 66.2% in case of commercial vaccine as demonstrated in Table 4 when chickens were challenged after single dose vaccination assay. At the same concerns, challenged birds after booster dose vaccination assay revealed a protective indices of 82.5%, and 79.5% corresponding to local and commercial imported batches respectively as shown in Table 4. This observation confirmed that the trivalent Salmonella vaccine could be more effective and more protective against natural and experimental infections with different types of salmonellae included in the vaccine preparation. Also the combination of the three different salmonellae either *Salmonella kentucky, Salmonella typhimurium* or *Salmonella enteritidis* does not interfere or affect the immune response of birds against each of them.[8]

Conclusion

Evaluation of the combined salmonella vaccine depending on protective indices is more obvious and the picture is better than evaluation depending on either measurement of humoral response or mortalities post challenge because of protective indices depends on several parameters reflecting the immune status of the birds including mortalities, clinical signs and post mortem lesions.

The trivalent *Salmonella* inactivated vaccine is vital in eliciting adequate antibody titers which aiding in conferring adequate immunity enable birds to pass experimental and so natural infections with the *salmonella* species. Also, the combination of *Salmonella typhimurium, Salmonella enteritidis* and Salmonella Kentucky vaccine proved to be of great value because of its high efficacy with a good and significant result in safety and protection.

Vaccination with the locally prepared polyvalent Salmonella inactivated vaccine could contribute protection and lower Salmonella prevalence in layers, breeders and in turn in broiler chickens.

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None.

Conflict of interest

Author declares that there is no conflict of interest.

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