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Preparation and evaluation of formalized bivalent Newcastle and *Salmonella* poultry vaccine

Sarah Sobhy[1*] ORCID: https://orcid.org/0000-0002-2535-3238
Mounir M. El-Safty[1] ORCID: https://orcid.org/0000-0003-1199-1375
Sherif Marouf[2] ORCID: https://orcid.org/0000-0003-4554-6974
Hala Mahmoud[1] ORCID: https://orcid.org/0000-0001-2462-956X
Jakeen EL-Jakee[2] ORCID: https://orcid.org/ 0000-0002-5299-3783

[1] Central Laboratory for Evaluation of Veterinary Biologics, Agriculture Research Centre, Cairo, Egypt.
[2] Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

Email: sarahsobhy@ymail.com

This study was conducted to prepare and evaluate the potency of different inactivated vaccine formulations that protect chickens against *Salmonella* Enteritidis and Newcastle disease virus using Montanide as adjuvant. Protection and the humoral immune response of prepared vaccines against *Salmonella* Enteritidis and Newcastle disease virus was evaluated and compared to imported vaccine. In this study, different formulae of *Salmonella* Enteritidis and Newcastle disease vaccines were prepared and compared with the imported one by measuring the antibody titer against Newcastle disease virus by hemagglutination inhibition test and the antibody titer against *Salmonella* Enteritidis using Enzyme Linked Immunosorbent Assay. On the other hand, the protection percentages against Newcastle disease and *Salmonella* Enteritidis were recorded to determine the best effective formula. The highest hemagglutination inhibition antibody level against NDV at first week was recorded for the prepared combined Newcastle disease and *Salmonella* Enteritidis vaccine (4.2 $\log_2$) followed by the prepared monovalent Newcastle disease (3.4 $\log_2$); the lowest antibody level (3.1 $\log_2$) was obtained with the imported vaccine. A gradual increase was observed in all groups to 7.1 $\log_2$, 6.8 $\log_2$ and 6.4 $\log_2$ at fourth week post vaccination, respectively. The antibody titer against *Salmonella* Enteritidis was 552 for the prepared combined *Salmonella* Enteritidis and Newcastle disease, followed by the prepared monovalent *Salmonella* Enteritidis (477) at first week post vaccination; the antibody titer obtained for the imported vaccine was 477. There was a gradual increase to 1456, 1406 and 1130 at fourth week post vaccination, respectively. Prepared combined vaccines gave the highest protection percentage, followed by prepared monovalent types and finally imported vaccines. Vaccination by the prepared combined *Salmonella* Enteritidis and Newcastle disease vaccine may be a way to increase the resistance of birds to S*almonella* and Newcastle and to decrease the shedding rate.

Keywords: hemagglutination inhibition test; ELISA; Newcastle disease virus; *Salmonella* Enteritidis; combined vaccines.

Introduction

Salmonella infections are considered the most important affecting poultry. The disease causes severe damage among young birds, with a high mortality rate. Adult birds are often chronic carriers of *Salmonella* without outward signs. The prevalence of *Salmonella* species in broiler flocks was 10.37% among three governorates in Egypt (Al-Qalyubia, El-Ismailia and El-Gharbia). The highest incidence of *Salmonella* was recorded in El-Gharbia, then El-Ismailia and finally Al-Qalyubia. The incidence of salmonella in closed system farms was higher than in open system farms. It was noticed that the

* Central Laboratory for Evaluation of Veterinary Biologics, Agriculture Research Centre, Cairo, Egypt.

most common *Salmonella* serotype was *Salmonella* Enteritidis (SE) followed by *Salmonella* Kentucky, *Salmonella* Newport, *Salmonella* Derby, *Salmonella* Typhimurium and *Salmonella* Infantis.[1]

Salmonellosis is an infectious disease of humans, animals and food production scale. It is responsible for more than 80 million cases all over the world. Historically, SE first was known as a significant public health disease in the 1980s.[2] Vaccination is the most appropriate approach to control the number of recorded cases. In Egypt, avian salmonellosis causes economic losses. About 51 strains of *Salmonella* were isolated in 2012; 19 (37.25%) serotypes were SE; 10 (19.60%) *S*. Infantis; 4 (7.84%) *S*. Kentucky; 1 (1.96%) *S*. Chiredzi; 15 (29.41%) S. Typhimurium and two (3.92%) *S*. Tsevie. In 2014, different *Salmonella* strains were detected in 5% of minced meat samples, 10% of burger samples, 35% of sausage samples and 25% of poultry products. The most identified *Salmonella* isolates were *S.* Infantis*, S.* Lagos*, S.* Bolombo*, S.* Cerro*,* SE*, S.* Kentucky*, S.* Newlands*, S.* Newport*, S.* Saintpaul*, S.* Sandiego*, S.* Senftenberg and *S.* Typhimurium*. Salmonella* vaccines, both live and inactivated, are effectively used to vaccinate chickens to decrease fecal transfusion, ovarian transmittance and within-flock prevalence. Inactivated vaccines are commonly used, because they do not have the reasonable public health threats.[2]

Newcastle disease (ND) is a destructive avian disease caused by an avian paramyxovirus type 1. It has a great pathogenicity variation in chickens and causes regular epizootics in Asia, Africa, Central America and South America. It can be grouped according to clinical signs into viscerotropic velogenic, neurotropic velogenic and mesogenic. Signs include mainly respiratory forms, nervous manifestation, and low mortality. During 2005, velogenic Newcastle disease virus (NDV) caused a major outbreak among commercial broiler chickens in Egypt.[3]

In Egypt, NDV is endemic and has caused serious economic losses since the first infection in 1948. NDV strains have two classes, I and II. Class I represents the avirulent strains, while class II has fifteen virulent genotypes I-XV and virulent vaccinal strains (LaSota, and Hitchner B1). Genotypes V, VI, and VII are the most circulating strains. The genotype VIId is the prevalence strain in Egypt and has caused different outbreaks in Egyptian poultry farms.[4]

The commercially available ND vaccines were developed based on NDV isolates genetically divergent from field strains that can only prevent clinical disease, but not shedding of virulent heterologous virus, highlighting the need to develop genotype-matched vaccines.[5]

The vaccination program and vaccine type differ according to the field virus virulence and the herd immune status. Vaccination against ND is mainly aimed at reducing and eliminating the clinical signs leading to low virus shedding and death after infection with virulent NDV. Vaccination with inactivated NDV vaccine may be advantageous due to the ability to prepare a wide range of low and high virulence NDV genotypes.[6] Montanide ISA70, a ready to use water in-oil adjuvant emulsion, is recommended for the preparation of avian vaccines.[4,6]

The purpose of the current work was to prepare and evaluate the potency of a bivalent Newcastle and *Salmonella* inactivated vaccine.

Materials and Methods

Preparation of *Salmonella* Enteritidis vaccine

A well-identified local strain of SE kindly obtained from the Strain Bank Department at the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Egypt, was used. Bulk culture was prepared and adjusted to produce a resultant final suspension of *S*E of 10^{10} CFU/0.5 mL. Formaldehyde solution 37% was added to the final *Salmonella* suspension at 0.2% with stirring at 24ºC for 24 h, then neutralized by sodium metabisulfite and stored at 5-7ºC. The inactivated culture (represented 500 doses) was centrifuged at 5000 rpm, 20 min at 4ºC and the bacterial cell pellet was collected. Montanide ISA70 was mixed to the prepared bacterin in a ratio of 30:70 to form an emulsion.[7]

Preparation of Newcastle disease virus vaccine[8]

NDV strain with a titer of $10^{10.5}$ Egg infective dose fifty (EID_{50})/mL and 10 $\log_2$ hemagglutination (HA) unit was

kindly provided by CLEVB. A formaldehyde solution (37%) was added to the NDV solution at 0.1%, then mixed and incubated at 37°C for 16 h. Montanide ISA70 adjuvant was added as a ratio of 1:1.

Preparation of bivalent *Salmonella* and Newcastle disease virus vaccine

The previously prepared inactivated SE and NDV were mixed together in equal volume.[9]

Imported vaccines

An imported inactivated ND vaccine with oil adjuvant (MEVAC^{TM} ND, Kemin Bilogicals, USA, 500mL) was administrated intramuscularly (IM) at a dose of 0.5 mL/ bird as recommended.

An imported inactivated *Salmonella* Enteritidis (Nobilis® SALENVAC T, MSD, UK; 500mL Bottle Dose 0.5 mL IM injection) was supplied by CLEVB and was used according to the manufacture recommended dose and rout.

Both vaccines were previously evaluated at CLEVB and proved to be potent.

Quality control of the prepared and imported vaccines[10]

To ensure that the *Salmonella* organisms were completely inactivated, Salmonella Shigella (S.S) agar medium was inoculated with the formalin-inactivated bacteria. After 24-48 h of incubation at 37°C, the absence of visible *Salmonella* growth after 3 passages indicated complete inactivation of the organism. The prepared and imported *Salmonella* vaccines were tested for freedom from any fungal contaminants by inoculation onto Sabouraud dextrose agar plates and incubation for 7 days at 25°C. In addition, the vaccines were inoculated on Pleuropneumonia like organism broth tubes and agar plates and were incubated at 37°C for 72 h and 14 days, respectively in CO_2 incubator to ensure the freedom of the vaccine from mycoplasma. Ten Specific Pathogen Free (SPF) chicks (3 weeks) were used for safety testing for each vaccine by injecting a double vaccination dose. The birds were observed for 14 days.

Also, complete inactivation of NDV was assessed using the vaccine virus infectivity test. Five of 9 days old SPF-embryonated chicken eggs (ECE)s were inoculated via allantoic cavity by 2/5 bird dose and were incubated at 37 ^{0}C for 7 days with daily candling for daily observation. At 7th day, eggs were kept at 4^{0}C for 24 h for HA test of collected allantoic fluids. The previous steps were repeated for a second passage. The vaccines were considered completely inactived if no HA activity was recorded, meaning that the vaccines have no residual infective virus.

Experimental design

Two hundred and seventy 3-weeks-old SPF chicks were obtained from SPF poultry farm at Koom Osheem-Fayoum province, Egypt. Chicks were divided into nine groups (30 chicks/each). Each group was housed separately in biosafety isolator to be vaccinated.

The experimental groups were named and divided (in correspondence with the immunization to be received) as follows: G1 (Prepared ND), G2 (Bivalent prepared ND and SE), G3 (Imported ND), G4 (Montanide ISA 70), G5 (Control), G6 (Prepared SE), G7 (Bivalent prepared ND and SE), G8 (Imported SE) and G9 (Montanide ISA 70).

Chicks in groups G1 (Prepared ND), G2 (Bivalent prepared ND and SE), G3 (Imported ND) and G4 (Montanide ISA 70) at 3 weeks old, received a single immunization by IM route at a dose of 0.5 mL, with the corresponding vaccine for each group.

Chicks in groups G6 (Prepared SE), G7 (Bivalent prepared ND and SE), G8 (Imported SE) and G9 (Montanide ISA 70) at 8 weeks old, received a single immunization by IM route at a dose of 0.5 mL, with the corresponding vaccine for each group.

Serum samples were collected weekly for 4 weeks and stored at -20^{o}C for serological tests.

Four weeks post vaccination all groups were challenged according to the vaccine used.

The SE vaccinated groups were challenged with the SE virulent strain using a challenge dose of 10^{8} CFU/mL each bird. Re-isolation of the challenge strain (shedding test) was performed according to.[9] All birds were culled 14 days post challenge. Samples (1g) from internal organs (heart, liver, and spleen) from chickens died during the challenge were examined bacteriologically.[11] Cloacal swab and organs were

cultured at 3rd, 5th, 7th, 10th and 14th days post challenge. The degree of protection was assessed according to the severity of the clinical signs, mortality rate, postmortem lesions and percentage of re-isolation.(12)

Newcastle vaccinated groups were challenged with virulent NDV type 7 NDV-B7-RLQP-CH-EG-12, accession No KM288609. NDV challenge dose titer was 10^6 EID_{50}/0.1 mL per bird. Cloacal and tracheal swabs were collected after challenge at 3rd, 5th, 7th, 10th and 14th days. The samples were diluted (10 fold) in PBS and inoculated into SPF eggs (9 days), incubated at 37°C and examined for mortality and hemagglutination inhibition (HI) for 5 days. (12) The degree of protection was assessed according to the severity of the clinical signs, mortality rate, postmortem lesions and HI test results. (13)

Measurement of post vaccination humoral immune response

Serum samples of groups G1 to G5 were tested against NDV using HI test and groups G6 to G9 were tested against SE using ELISA test (ID Screen Avian Salmonella Indirect Groups B and D, Innovative Diagnostics- ID vet kit, Francia), indirect ELISA for the detection of antibodies against anti- *Salmonella* Groups

$$S/P = \frac{OD\ sample - OD\ NC}{ODPC - ODNC}$$

B and D in chicken serum.

The test procedure was performed according to the manufacture instructions and interpretation for each sample was calculated as follows:

NC: negative control. PC: positive control. OD: optical density

Antibody titer:

Log_{10} (titer) = 1.00 x Log_{10} (S/P) + 3.4

S/P value: ≥ 0.3, ELISA Antibody titer: ≥ 754, *Salmonella* immune response is positive.

Ethical approval

All work has been applied according to the international biological animal welfare instructions.

Results

The imported and prepared vaccines were proved to be sterile, safe and completely inactive after third passage.

At first week post vaccination, the SE antibody titers induced in chickens immunized with the prepared *Salmonella* and bivalent Newcastle *Salmonella* vaccines, as well as with the imported *Salmonella* vaccine, determined by ELISA, were 477, 552 and 477, respectively, while in the unvaccinated control group it was 404. Moreover, a gradual increase was shown until reach 1406, 1456 and 1130, respectively, at 4th week post vaccination. Nevertheless, the recorded titer at 4th week (389) for the control unvaccinated group was near the recorded titer at first week (404) (Table 1).

HI titers against NDV were recorded weekly for (G1, G2, G3, G4 and G5). The HI titers against NDV of chickens vaccinated with prepared Newcastle, bivalent Newcastle SE and imported Newcastle vaccines were 3.4, 4.2 and 3.1, respectively, at first week post vaccination; while it was zero in the unvaccinated control group. Moreover, a gradual increase was shown to reach 6.8, 7.1 and 6.4, respectively, at 4th week post

Table 1. Humoral immune responses against *Salmonella* Enteritidis in chickens using ELISA test to determine *Salmonella* antibody titers.

Groups	Weeks post vaccination/ *Salmonella* antibody titers log_{10}			
	1st week	2nd week	3rd week	4th week
G6	477±8.8	703±13	979±18	1406±26
G7	552±10	778±14	1054±19	1456±27
G8	477±8.8	602±11	803±14.8	1130±21
G9	329±6	308±5.6	276±5	248±4.5
G5	404±7.5	399±7.4	396±7.3	389±7

G6: Prepared SE. G7: Bivalent prepared ND and SE. G8: Imported SE. G9: Montanide ISA 70. G5: Control.

Table 2. Humoral immune responses against Newcastle Disease virus in chickens using HI (log2) test.

Groups	Weeks post vaccination/ HI (log_2) test			
	1st week	2nd week	3rd week	4th week
G1	3.4 ± 0.2	4.8 ± 0.3	6.0 ± 0.4	6.8 ± 0.4
G2	4.2 ± 0.3	5.4 ± 0.4	6.8 ± 0.5	7.1 ± 0.6
G3	3.1 ± 0.2	4.2 ± 0.3	5.8 ± 0.4	6.4 ± 0.4
G4	0	0	0	0
G5	0	0	0	0

G1: Prepared ND. G2: Bivalent prepared ND and SE. G3: Imported ND. G4: Montanide ISA 70. G5: Control.

Table 3. Protection rates of vaccinated chickens after challenge with *Salmonella* Enteritidis virulent strain.

Groups	Mortality number (n=20)	Protection (%)
G6	5	75
G7	4	80
G8	6	70
G9	17	15
G5	18	10

G6: Prepared SE. G7: Bivalent prepared ND and SE. G8: Imported SE. G9: Montanide ISA 70. G5: Control.

Table 4. Re-isolation rates of *Salmonella* Enteritidis from liver, heart blood and spleen in vaccinated and control groups.

Groups	Swab (n=10)	Organs (n=10)			Total	
	Positive	Liver	Heart	Spleen	Positive/total	%
G6	0	0	0	0	0/40	0
G7	0	0	0	0	0/40	0
G8	2	0	0	0	2/40	5
G9	8	9	7	7	31/40	77.5
G5	7	8	6	7	28/40	70

G6: Prepared SE. G7: Bivalent prepared ND and SE. G8: Imported SE. G9: Montanide ISA 70. G5: Control.

vaccination. However, in the unvaccinated control group, the reported titer was the same (0) (Table 2).

Protection percentages of chickens vaccinated with prepared SE vaccines and the imported SE one at 4 weeks after challenge is shown in Table 3. Protection was 80% in the group G7 (vaccinated with the prepared bivalent vaccine), 75% in the group G6 (vaccinated with the prepared *Salmonella* Enteritidis vaccine), 70% in the group G8 (vaccinated with the imported one), while it was 15% for the group inoculated with adjuvant (G9) and 10% for the unvaccinated group (G5).

SE re-isolation (recovery test) from liver, heart blood and spleen is shown in Table 4. It was noticed that re-isolation of SE from liver, spleen and heart of chickens vaccinated with the prepared *SE* and the bivalent one was 0% at 4 weeks post challenge. Re-isolation was 5% for the imported vaccine. On the other hand, for the group inoculated with the adjuvant re-isolation was 77.5

Table 5. Frequency of NDV isolation (shedding test) of challenged control and vaccinated groups.

Groups		Number of positive ND swab/day n=5			
	Swabs	3 days	5 days	7 days	10 days
G1	T	4/5	0/5	0/5	0/5
	C	4/5	0/5	0/5	0/5
G2	T	3/5	1/5	0/5	0/5
	C	3/5	1/5	0/5	0/5
G3	T	0/5	0/5	0/5	0/5
	C	0/5	0/5	0/5	0/5
G4	T	5/5	5/5	N/A	N/A
	C	5/5	5/5	N/A	N/A
G5	T	5/5	5/5	N/A	N/A
	C	5/5	5/5	N/A	N/A

n: number of chickens. G1: Prepared ND. G2: Bivalent prepared ND and SE. G3: Imported ND. G4: Montanide ISA 70. G5: Control. T: Tracheal swabs. C: Cloacal swabs. N/A: not applicable.

%, a higher percent than the recorded for the unvaccinated group (70%).

The NDV shedding was judged by collecting tracheal and cloacal swabs from all experiment groups at 3rd, 5th, 7th and 10th day post challenge. On day 3 the shedding was zero for the group vaccinated with the imported vaccine (G3). The viral shedding was zero on the 5th day for groups vaccinated with the prepared ND (G1) and the imported vaccine (G3), while the group vaccinated with the bivalent vaccine (G2) recorded zero shedding on the 7th day post challenge (Table 5). Either the group inoculated with the adjuvant or the unvaccinated one showed 100% shedding until day 5, then died completely with clear ND symptoms.

Discussion

Salmonella infection is a critical veterinary and medical problem worldwide and a major issue in the food industry. *Salmonella enterica* is a zoonotic bacteria transmitted through the food chain and an important cause of disease in humans.[14]

There are several methods to control salmonellosis in poultry and decrease its economic losses and other problems; among these methods are the use of vaccines, promising antibiotics, organic acids, essential oils, cinnamaldehyde and chitosan, nanoparticles.[15]

ND and salmonellosis are very crucial diseases in the world including Egypt. Both of them, can cause clearly perceptible economic losses in poultry production. Prevention of such diseases needs production of valuable vaccines. ND-*Salmonella* bivalent vaccine can be considered a helpful choice to deal with this problem.[16] Unfortunately, bivalent ND-*Salmonella* vaccine is not locally produced yet in Egypt, so it is of considerable importance to develop the bivalent formula of the ND-*Salmonella* vaccine to overcome ND as well as salmonellosis.

Both, NDV and *Salmonella* strain used for vaccine preparation process was locally isolated from infected chickens. This study includes a comparison of three formulae of prepared *Salmonella* and ND vaccines in addition to the imported one. Using *Salmonella* in combination with NDV has a well-known significance in vaccines production, the ability to elicit the body's immune system to resist *Salmonella* infection can also significantly enhance the immune effect of the exogenous gene being carried.[17] The inactivated *Salmonella* vaccine has the ability to induce immune protection in poultry and decrease the rate of colonization. The prepared vaccine contains a number of antigens that are expressed in the very earliest phases of infection. Additionally, some genes that encode certain important antigens like flagella and lipopolysaccharide were down-regulated in the chickens' intestinal tract.

The present work was designed to study the immunological effect of different prepared formulae of formalized inactivated SE and ND vaccine in chickens

and their capability to protect chickens from getting infection. Serological tests as ELISA and HI were performed to determine the antibody responses against SE and NDV in chicken sera, respectively. Moreover, challenge test was applied to determine protection percentage and shedding rate of both *Salmonella* and Newcastle after challenge of vaccinated birds with monovalent and bivalent forms of the prepared vaccines.

Concerning the results of serological tests, ELISA for *Salmonella* showed that the group immunized with the bivalent ND and SE vaccine (G7) had the highest antibody titer (552), followed by the G6 group that received the prepared SE vaccine (477); at the same time, the G8 group vaccinated with the imported monovalent SE vaccine registered (477) at first week post vaccination. These results can be owed to the effect of combination of SE and NDV in a bivalent vaccine that is known to have an enhanced effect on the immune response to SE to be induced in the vaccinated chickens of the group 7. These results were previously explained by[18] who supported the approach of using prepared bivalent vaccine to stimulate protective level of SE antibodies for four weeks after vaccination. The administration of the bivalent SE/ND vaccine evokes increased quantities of antibody against SE component of the vaccine, referring it to be more successful and economic.

The aforementioned results were proved for the ND vaccinated groups, as the bivalent formula gave the highest antibody titer in the whole experiment compared to the groups vaccinated with the monovalent vaccine, either prepared or imported. Group 2, which was vaccinated by the bivalent SE/ND vaccine, recorded the HI titer of 7.1, while the other groups recorded 6.8 and 6.4 for the prepared and imported vaccine, respectively.

Here it could be said that the bivalent vaccination strategy is able to increase responsiveness for different pathogens like, *Salmonella* and Newcastle**.** Moreover, bivalent vaccine formulations obviously expand the extent of a single vaccine formulation. Work was followed to determine and compared the protective efficacy of the previous different vaccine formulae by calculating the protection percentages of different vaccinated groups after experimental challenge and also the shedding percentage was estimated to fulfill all the comparison points.

The protective efficacy of tested vaccines showed that the bivalent SE/ND vaccine could protect chickens despite having lower protection than the ND vaccinated group. This improved immune efficacy was observed when bivalent vaccines were compared to monovalent ones as measured by the antibody titer. These results agree with previous studies which supported the concept of using bivalent NDV as a valuable element in poultry vaccination strategy.[18]

The study illustrates a comparison between different formulae of inactivated Newcastle and SE. The tested vaccines evoke different levels of protection against both Newcastle disease and salmonellosis; in other words, they could decrease the bacterial load of SE in vaccinated birds forming a considerable percentage of protection that ranged from 70% to 80%, according to the vaccine formula.[19]

The above discussed results are in accordance with an earlier research published by[20] who stated that the inactivated *Salmonella* vaccine is considered satisfactory if the protection percentage is 70% or more and not exceed 20% for control birds.

On the other hand, caecal colonization was assessed by cloacal swabbing and re-isolation of *Salmonella* strain from heart blood, liver and spleen to estimate the bacterial recovery post challenge of the vaccinated groups to show a low shedding percentage for the groups vaccinated by the bivalent prepared vaccine. The results obtained for monovalent prepared vaccines come in match with other studies.[18] The bivalent prepared vaccine showed lower degree of shedding and re-isolation, which is correlated with the caecal colonization of *Salmonella.* This phenomenon is well known and is probably associated with intermittent caecal evacuation.[18] In addition, the protective effect of bivalent prepared vaccine was assessed for their effect in chickens against colonization and systemic invasion. It confirms that the response was satisfactory to stop the caecal colonization among the bivalent (ND/SE) vaccinated group after challenging with avirulent SE. In another study, 4.2% of re-isolation was reported for the monovalent prepared *Salmonella* vaccine, which is

lower than it was recorded in this study. This confirms the improved results of the bivalent vaccine compared to the monovalent one.[18]

In the present work, the group G2 (vaccinated with bivalent ND and SE) gave higher titer than G1 (prepared ND) and G3 groups (imported vaccine). It was observed that vaccination with a bivalent vaccine containing Montanide ISA70 adjuvant provides a higher immune response against ND antigens compared to a monovalent vaccine. In a study conducted by[21] was proved that the bivalent NDV vaccines have the ability to increase NDV antibody titers. Peak titers of HI antibodies were observed at 4th week post vaccination for all vaccines; the mean HI titer of 7.1 log_2 was obtained for the group vaccinated with the bivalent prepared ND/*Salmonella* (G2);6.8 log_2, for the group vaccinated with the monovalent ND vaccine (G1) and 6.4 log_2, for the group vaccinated with the imported one (G3).

The results of the current study come in agreement with other study[22] which recommended the use of the bivalent prepared vaccine to reduce *Salmonella* infection as well as NDV infections and recorded the increased protective antibody titer against ND and SE.

Conclusion

Our results suggested that the inactivated bivalent NDV and SE vaccine could induce a satisfactory immune response in vaccinated chickens with proper protection efficacy against both NDV and SE. The prepared vaccine could therefore potentially be applied in the poultry industry to control and prevent ND and SE in chickens in Egypt. Additionally, the results indicated that using of bivalent prepared vaccine is recommended to save time and economic on the production scale and finally it can reduce the stress of vaccination for chickens.

Conflict of interest

The authors declare that there is no conflict of interest.

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Author's contributions

Sarah Sobhy: applied the experiment and wrote the manuscript.

Mounir M El-Safty: experimental design.

Sherif Marouf: applied the experiment.

Hala Mahmoud: applied the experiment.

Jakeen EL-Jakee: wrote the manuscript.

All authors reviewed and approved the final version of this manuscript for publication.

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Preparación y evaluación de una vacuna aviar bivalente contra Newcastle y *Salmonella*, inactivada con formaldehído

Resumen

Este estudio se llevó a cabo para preparar y evaluar la potencia de diferentes formulaciones de vacunas inactivadas que protegen a los pollos contra *Salmonella* Enteritidis y el virus de la enfermedad de Newcastle utilizando Montanide como adyuvante. Se evaluó la protección y la respuesta inmune humoral de las vacunas preparadas contra *Salmonella* Enteritidis y el virus de la enfermedad de Newcastle y se comparó con la vacuna importada. En este estudio se prepararon diferentes fórmulas de vacunas contra *Salmonella* Enteritidis y la enfermedad de Newcastle y se compararon con la importada midiendo el título de anticuerpos contra el virus de la enfermedad de Newcastle mediante la prueba de inhibición de la hemaglutinación y el título de anticuerpos contra *Salmonella* Enteritidis mediante ELISA. Por otra parte, se registraron los porcentajes de protección contra la enfermedad de Newcastle y *Salmonella* Enteritidis para determinar la fórmula más eficaz. El mayor nivel de anticuerpos inhibidores de la hemaglutinación contra el virus de la enfermedad de Newcastle, en la primera semana, se registró con la vacuna combinada preparada contra la enfermedad de Newcastle y *Salmonella* Enteritidis (4,2 log2), seguida de la vacuna monovalente preparada contra la enfermedad de Newcastle (3,4 log2); el menor nivel de anticuerpos (3,1 log2) se obtuvo con la vacuna importada. Se observó un aumento gradual en todos los grupos hasta alcanzar 7,1 log2, 6,8 log2 y 6,4 log2 en la cuarta semana tras la vacunación, respectivamente. El título de anticuerpos contra *Salmonella* Enteritidis fue de 552 para la vacuna combinada preparada contra la *Salmonella* Enteritidis y enfermedad de Newcastle, seguida por la vacuna monovalente preparada contra *Salmonella* Enteritidis (477) en la primera semana después de la vacunación; el título de anticuerpos obtenido con la vacuna importada fue de 477. Hubo un aumento gradual hasta 1456, 1406 y 1130 en la cuarta semana después de la vacunación, respectivamente. Las vacunas combinadas preparadas dieron el mayor porcentaje de protección, seguidas por los tipos monovalentes preparados y, por último, por las vacunas importadas. La vacunación con la vacuna combinada preparada contra la *Salmonella* Enteritidis y la enfermedad de Newcastle puede ser una forma de aumentar la resistencia de las aves a la *Salmonella* y Newcastle y de disminuir la tasa de excreción ion.

Palabras clave: pruebas de Inhibición de Hemaglutinación; ELISA; virus de la enfermedad de Newcastle; *Salmonella* Enteritidis; vacunas combinadas.

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