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RESEARCH

Shedding pattern of classical and variant strain of IB challenge virus in SPF and commercial layer vaccinated chicken with trivalent vaccine containing H9N2 AI, LaSota, classical M41 and var 2 IB viruses at 1, 3 and 7 months post vaccination

Hanan Sayed [1], Mohamed S. Madkour [1], Hussein A. Hussein [2], Ahmed A El-Sanousi [2]

ABSTRACT

Background: Prolonged immune protection for classic and variant IBV in layer chicken rearing is an important issue.
Objective: Evaluation of the protection duration for IBV (M41 and Var2) post vaccination by inactivated combined (H9N2, LaSota, M41 and Var2 IB) vaccine based on laboratory tests and repetition of challenge test.
Methods: We prepared formalin inactivated combined vaccine for Avian Influenza (H9N2N), Newcastle (La), and IB (M41 and Var 2) by using montanide 71 adjuvant. The prepared vaccine was injected intramuscularly in specific pathogen free and commercial layer chicken, the injection was repeated as a booster vaccination after a month from the first vaccination. Humoral immune response for IBV was measured by ELISA weekly until about one month after the booster vaccination then periodically till the end of duration. Challenge trial for both IB strain was repeated after a month from the first vaccination and after two and six months from the booster vaccination.
Results: The use of formalin inactivated combined H9N2, LaSota and IB (M41 and Var 2) vaccine by using montanide 71 adjuvant via intramuscular vaccination enhanced the immune response as indicated by ELISA for IB, also induced protection until six months after the booster vaccination against challenge with IB (Var 2 and M41) viruses.
Conclusion: Usage of formalin inactivated combined H9N2, LaSota and IB (M41 and Var 2) vaccine with montanide 71 adjuvant can protect chicken until six months from the booster vaccination and decrease shedding of IBV.

Keywords: M41, Var2 IB; SPF chicken; layer chicken.

BACKGROUND

Infectious bronchitis (IB) is an acute highly contagious respiratory and nephropathogenic disease of chicken. Other signs as decrease in egg production and quality may occur (Gorgyo *et al.*, 1984; El-Mahdy *et al.*, 2010; Balasubramaniam *et al.*, 2013). The first isolations of infectious bronchitis virus (IBV) were in 1930s (Cheever *et al.*, 1949), then appeared at 1931 in United States of America (USA) by (Schalk and Hawn, 1931), from this date until now it continues to affect chicken of all ages and types in all parts of the world. Infection of IBV was reported for the first time in Egypt in 1950 (Ahmed, 1954). In Beni-Seuf in 1998, a new IBV strain was isolated from chick suffering from respiratory and renal symptoms and the S1 sequence of it was closely related to new Israeli strain (Abdel-Moneim *et al.*, 2002). From 2003 for two years, Twenty-five isolates were isolated from 13 Egyptian governorates (Mahgoub *et al.*, 2010; Abdel-Sabour *et al.*, 2017).

There are many serotypes of IBV due to amino acid changes in the S1 genome of the virus (El-Mahdy *et al.*, 2010). The main problem in IB vaccine that there is no cross-protection

Correspondence: sidovet@yahoo.com
Full list of author information is available at the end of the article

against antigenically unrelated serotypes and variant strains of the virus, so outbreaks can occur in vaccinated population (Gelb *et al.,* 1991; Capua *et al.,* 1994; Jia *et al.,* 1995).

Practically, combined vaccines are less stressful for both the worker and the bird (especially laying hens) than separate monovalent vaccine (Ali *et al.,* 2017).

Inactivated vaccines are safe, effective, relatively inexpensive and individual inoculation can ensure high coverage within the vaccinated chicken but it needs individual application. The ideal vaccine adjuvants must enhance the immune response and should be stable and safe for environment and chicken (Lone *et al.,* 2017).

In this study, we prepared inactivated combined vaccine for H9N2, NDV LaSota strain and IBV (Var 2 and M41). We also evaluated its immunogenicity and protective efficacy for both IBV strains up to six months from the booster vaccination through ELISA test and repetition of both IBV strains challenge.

MATERIALS AND METHODS

SPF ECEs

Eggs were obtained from Nile SPF Farm, Kom Oshiem, Fayom, Egypt, and used for virus propagation, virus titration, and assurance of complete inactivation (OIE, 2018).

Antigen

H9N2: (A/chicken/Egypt/D4692A/2012), NDV: LaSota and IBV: Var2 (IBV-S1/VSVRI_G9/Egy 013) with accession Number (KP729422) (sequence of S1 gene) and M41. All viruses were kindly provided by Department of Poultry Vaccines Production Unit, Veterinary Serum and Vaccine Research Institute, Abbasia, Egypt.

Characterization of the viruses

Characterization of the viruses was made by National Laboratory for Veterinary Control on Poultry Production, Animal Health Research Institute, Egypt. Real-time (RT) PCR was made to H9N2 sample to identify the virus and make sure it is free from H5N1, ND or IB viruses. Identification of LaSota, IB M41 and variant IB viruses was made by HI test by reference antigen, RT-PCR and sequence analysis respectively.

Viruses' propagation and titration

Viruses were propagated in specific pathogen-free (SPF) embryonated chicken egg (ECE). The obtained harvest from each virus was titrated in SPF ECEs and calculated according to a method of (Reed and Muench, 1938).

Formalin inactivator

Formalin in a final concentration of 0.1% of the total volume was used for viruses' inactivation. Samples from each inactivated virus were tested for complete inactivation in 10-day-old SPF ECE for two successive blind passages to insure the absence of residual live virus.

Adjuvant

Montanide™ ISA 71 VG mineral oil based adjuvant was used in this study to make water-in-oil (W/O) emulsion.

Vaccine formulation

A ratio of 30/70 (v/v) aqueous/oil was prepared. Stable preparations are obtained by mixing the aqueous medium into the Montanide™ ISA 71 VG, at room temperature or less, under vigorous stirring (for 15-30 min) as recommended by SEPPIC Co; the manufacture Co.

Evaluation of humoral immune response to IBV

It was carried by ELISA test.

Challenge trial

SPF and Commercial layer 21 days old chicken are divided into two groups, one group was vaccinated with 1 ml via intramuscular route from the prepared combined vaccine; other group was kept non-vaccinated as control negative group. After 4 weeks post vaccination, 13 birds from each group were challenged with 10^4 EID_{50} IB M41 virus/bird and 13 birds from each group were challenged with 10^4 EID_{50} Var 2 IB virus/bird, both via intranasal and intraocular route. Oropharyngeal swabs at 3, 5, 7 and 10 DPC were collected from challenged groups and tested for virus shedding by real-time PCR.

Intramuscular 1 ml booster vaccination was given to the vaccinated group after one month from the first vaccination, then we Repeat the challenge typically as the first time twice, after two and six months from the booster vaccination.

Serum samples were collected weekly until one-month post booster vaccination then monthly for detection of serum Abs for IBV by ELISA test.

RESULTS

Serological response

ELISA test for IBV

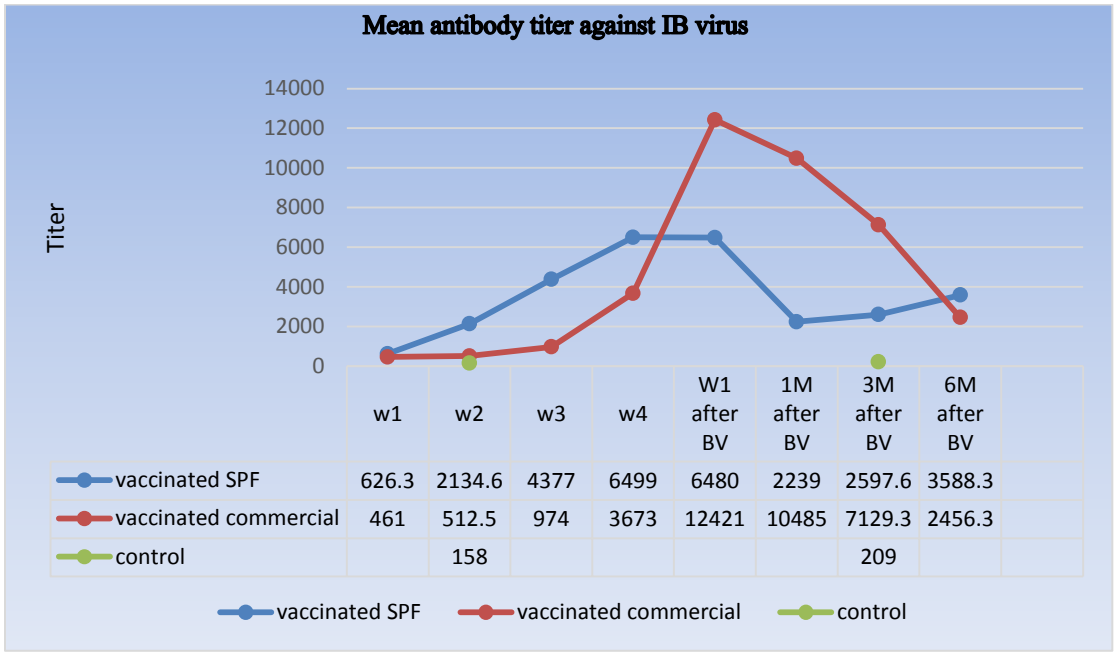

Fig. 1: mean antibody titer against IBV by ELISA test. BV: booster vaccination, control group not vaccinated by any vaccine

Table 1: The mean ELISA titer ± SD of IB virus for SPF and commercial chicken after the first vaccination.

Chicken type	Weeks after vaccination			
	1	2	3	4
SPF	626.3±247[a]	2134.7± 687[a]	4377± 4604[a]	6499± 3388[a]
Commercial	461±265.7[b]	512.5±124[a]	974±174.8[a]	3673± 2326.6[a]

Means ELISA titer with different letters (a, b) within the same column are significantly different at P value ≤ 0.05 between chicken groups

Table 2: The mean ELISA titer ± SD of IB virus for SPF and commercial chicken after the booster vaccination.

Chicken type	Time after booster vaccination			
	1 week	1 month	3 month	6 month
SPF	6480±5882[a]	2235.2± 2384[a]	2597.6± 2527[a]	3588.3± 1469[a]
Commercial	12420.7±8687[a]	10484.7±9657.8[a]	7129.3±467.2 [a]	2456.3± 2206[a]

Means ELISA titer with different letters (a, b) within the same column are significantly different at P value ≤ 0.05 between chicken groups

Shedding ratio after challenge with variant IB virus in SPF and commercial chicken

Oro-pharyngeal shedding after challenge with variant IB for vaccinated and positive control groups at interval days was measured by real-time RT-PCR. It was observed that the control groups had the highest virus shedding values. All groups showed no mortalities. In the first SPF challenged chicken there is shedding in 5th and 7th day after challenge, very low shedding titer in the 3rd day while no shedding detected in the 10th day. In the second challenge, there is low shedding titer in the 3rd and 10th day while no shedding in the 5th and 7th DPC. In the third challenge, there is low shedding titer in the 3rd and 10th day, no shedding in the 5th day while high shedding titer in the 7th day, table (3). In the first commercial chicken challenge there is shedding in all interval days but very low shedding titer in the 10th day. In the second challenge there is shedding in 3rd day only. In the third challenge there is shedding in the 3rd and 10th day while no shedding in the 5th and 7th DPC, table (4)

Table 3: shedding ratio after challenge with var 2 IB virus in SPF chicken.

groups	3 DPC		5 DPC		7 DPC		10 DPC	
	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})
1st challenge	+ve	3.37	+ve	$4.878X10^3$	+ve	$2.580X10^3$	-ve	-
2nd challenge	+ve	٢.٣٦	-ve	-	-ve	-	+ve	$2.118X10^1$
3rd challenge	+ve	$8.632X10^1$	-ve	-	+ve	$2.517X\ 10^5$	+ve	$2.157X10^2$
Control +ve	+ve	$1.166X10^2$	+ve	$1.538X10^5$	+ve	$9.201X\ 10^4$	+ve	$8.632X10^1$

EID_{50}=Embryo infective dose 50% control +ve: challenged not vaccinated chicken DPC: day post challenge

Table 4: Shedding ratio after challenge with var 2 IB virus in commercial chicken.

groups	3 DPC		5 DPC		7 DPC		10 DPC	
	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})
1st challenge	+ve	$1.829X10^1$	+ve	$1.207X10^3$	+ve	$7.356X\ 10^3$	+ve	٣.٣٥
2nd challenge	+ve	$3.004X10^1$	-ve	-	-ve	-	-ve	-
3rd challenge	+ve	$1.741X10^1$	-ve	$9.921X10^4$	-ve	-	+ve	$3.422X10^2$
Control +ve	+ve	$1.166X10^2$	+ve	$1.538X10^5$	+ve	$9.201X\ 10^4$	+ve	$8.632X10^1$

EID_{50}=Embryo infective dose 50% control +ve: challenged not vaccinated chicken DPC: day post challenge

Shedding ratio after challenge with IB M41 virus in SPF and commercial chicken

The shedding in oro-pharynx after challenge with IB M41 virus for vaccinated and positive control groups at interval days was measured by real-time RT-PCR. It was observed that the control groups had the highest virus shedding values; there is no shedding in the first and second SPF chicken challenge in all interval days while in the third challenge there is no titer detected in the 10^{th} day only. There is no shedding in all interval days in all commercial challenged chicken except the 3^{rd} DPC after the second challenge only. All groups showed no mortalities, table (5, 6 and 7)

Table 5: Shedding ratio after challenge with M41 IB virus in SPF chicken.

groups	3 DPC		5 DPC		7 DPC		10 DPC	
	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})
1st challenge	-ve	-	-ve	-	-ve	-	-ve	-
2nd challenge	-ve	-	-ve	-	-ve	-	-ve	-
3rd challenge	+ve	2.05	+ve	8.75	+ve	7.88	-ve	-
Control +ve	+ve	$1.126X10^{2}$	+ve	$2.586X10^{1}$	+ve	$1.823X10^{1}$	+ve	1.66

EID_{50}=Embryo infective dose 50% control +ve: challenged not vaccinated chicken DPC: day post challenge

Table 6: Shedding ratio after challenge with M41 IB virus in commercial chicken.

groups	3 DPC		5 DPC		7 DPC		10 DPC	
	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})	result	Shedding amount (EID_{50})
1st challenge	-ve	-	-ve	-	-ve	-	-ve	-
2nd challenge	+ve	$2.686X10^{1}$	-ve	-	-ve	-	-ve	-
3rd challenge	-ve	-	-ve	-	-ve	-	-ve	-
Control +ve	+ve	$1.126X10^{2}$	+ve	$2.586X10^{1}$	+ve	$1.823X10^{1}$	+ve	1.66

EID_{50}=Embryo infective dose 50% control +ve: challenged not vaccinated chicken DPC: day post challenge

Table 7: Mean ELISA titer for both strain of IBV by ELISA post the first challenge in commercial chicken and the second challenge in SPF chicken.

	Mean AB titter in ELISA test for the second SPF chicken challenge				Mean AB titter in ELISA test for the first commercial chicken challenge			
	Var 2 challenged group		M41 challenged group		Var 2 challenged group		M41 challenged group	
	1WPC	2WPC	1WPC	2WPC	1WPC	2WPC	1WPC	2WPC
vaccinated	4364	3015	3747	6023	1569	7354	1716	2368
Control positive	654	------	571	608	------	------	------	------

WPC=Week post challenge control +ve: challenged not vaccinated chicken

DISCUSSION

It is very difficult to apply many injectable vaccines in chicken farms because of the huge number of chicken they contain compared to large animals (Abodalal, 2017), so the combined vaccines are suitable for these farms.

In this study, we reported the serological and the challenge response up to six months after booster vaccination to simulate the long rearing period in layer farms. We prepared combined inactivated oil emulsion vaccine for AIV H9N2, NDV and IBV (Var 2 and M41) with montanide 71 adjuvant. The humoral immune response was measured by ELISA test for IB virus.

The humoral immune response for IBV was investigated by ELISA test as shown in Figure (1) and table (1, 2). Mean antibody titer in SPF vaccinated chicken increased gradually to reach the highest titer between (6499) and (6480) at the fourth week after first vaccination and first week after booster vaccination, and reach (3588.3) at the 6th month after booster vaccination. While mean antibody titer in commercial vaccinated chicken increased to reach the highest titer (12422) at the 1st week after BV, and then decrease gradually to reach (2456.3) at the 6th month after BV.

Challenge tests for classical M41 and var 2 IBV were repeated at month after vaccination, two months and six months after booster vaccination. Results showed mild clinical signs in control positive group of the both strains of IBV, at the same time, 100% of the vaccinated chicken were protected from mortality and showed no clinical signs. Shedding test was carried out at the 3, 5, 7 and 10 DPC by oropharyngeal swabs by real time PCR revealed that this vaccine decreased the shedding of IBV compared to positive control group, this agrees with OIE (2012). Sera from the control positive group were negative by ELISA test post the second SPF chicken and the first commercial chicken challenge Table (7).

CONCLUSION

The prepared combined vaccine induced humeral immunity for IBV (M41 and var 2) and reduced viral shedding after challenge, it still protective for six months after booster vaccination.

AUTHOR DETAILS

[1]Veterinary Serum and Vaccine Research Institute, Abbasya, Agricultural Research Center, Cairo, Egypt.
[2] Faculty of Veterinary Medicine, Cairo University, Egypt.

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