The effect of chicken infectious anemia virus infection and infectious bursal disease vaccination on the efficacy of Newcastle disease virus vaccines

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ABSTRACT

A case-control study was performed to determine the effect of chicken infectious anemia virus (CIAV) as a risk factor associated with Newcastle Disease Virus (NDV) vaccination failure. Three groups each of 80 specific pathogen free (SPF) one day old chicks were used. The first group was infected with CIAV at one day old, the first and second groups were vaccinated with intermediate plus infectious bursal disease (IBD) vaccine at 10 days of age. All three groups were vaccinated with Hitchner B1 vaccine at 6 days of age. At 18 days of age, 40 birds of each group received LaSota vaccine, and the other 40 chickens received inactivated monovalent NDV vaccine by intramuscular (I/M) route. Ten days post live NDV vaccination, signs of depression, severe respiratory manifestation and mortalities occurred in CIAV-IBD-LaSota infected birds in comparison with other two control groups. Three weeks post NDV vaccination, retarded body weights were noticed in CIAV infected group only. The protection rates were decreased against virulent NDV from 100% in the 3rd group to 40% in CIAV infected groups.

INTRODUCTION

Since 2003, we have received complains from various parts in Egypt, chicken vaccinated with live and/or inactivated NDV vaccines, have shown signs of NDV, respiratory signs, nervous manifestation, decreased feed intake and increased mortality rates, following live NDV vaccination, begins at the age of 4 weeks in broiler flocks till 11 weeks in replacing pullets. The farm managers attributed this problem to the pathogenicity of NDV vaccines used, or a proposed vaccine failure. To investigate this proposal, it was suggested that presence of other chicken pathogens, may create the problem, for example reticuloendotheliosis virus (REV), CIAV, avian leucosis virus (ALV), or avian influenza virus (AIV). It was decided to set up two experiments. The first involved serological monitoring of ND antibody titre, any immuno-suppressive factor in the affected flock, retesting the NDV vaccine batches used in the vaccination of affected flock for pathogenicity using index of neurovirulence and mean death time (MDT) tests and potency test. Second experiment involved inquiring the same vaccination program in SPF chickens and evaluation the response to NDV vaccines.

MATERIAL AND METHODS

Eggs

Nine-eleven days embryonated chicken eggs originating from SPF flock routinely submitted to our laboratory from Kome Oshiem Farm, were used for estimation of mean death time (MDT) of live lentogenic LaSota vaccines and titration of LaSota and intermediate plus type of infectious bursal disease (IBD) vaccines.

Chickens

Chickens were hatched from SPF eggs of the same flock, their sera were monthly monitored in our laboratory for evidence of any of list of viral and bacterial infections among which CIAV antibodies.

Vaccines

The trade names of the vaccines used are not mentioned to avoid commercialism.

Commercial LaSota vaccine

An European commercial vaccine was reconstituted and used by ocular application at age of 18 days at rate of 10^7 EID_{50}/bird.

Commercial Hitchner B1 vaccine

Applied by eye dropping at 6 days of age after reconstitution at rate of $10^{6.5}$ EID_{50}/dose.

Inactivated NDV vaccine:

Commercial inactivated oil emulsion NDV vaccine containing 70 PD_{50}/dose (0.5 ml/bird) was used by IM at age of 18 days.

IBD vaccine

An intermediate plus IBD vaccine was used after reconstitution to contain $10^{2.7}$ EID_{50}/bird by eye dropping at age of 13 days.

Virulent virus strains

Newcastle disease virus (NDV)

Sheble and Reda velogenic viscerotropic NDV (unpublished data) was used to estimate the protection for NDV after vaccination. It was applied by IM as 10^6 EID_{50}/bird. The protection test was valid by 100% mortalities (within 6 days post challenge) of 10 non-NDV vaccinated chickens of the same age of challenged birds.

Chicken Infectious Anemia Virus (CIAV)

Virulent CIAV CUX-1 strain was obtained from Intervet, Boxmeer Company. The virus had been propagated in MDCC-MSB1 cells. The virus titration was performed *(1)* by serial dilution of the virus sample inoculated into 96 well microplate, that contain 4 x 10^4 MDCC-MSB1 cells and subcultured seven times to obtain the final titre. The virus was applied by IM route at one day old with 10^6 $TCID_{50}$/bird. The virulence of CIAV was indicated by anemia produced in infected chickens 2 weeks post inoculation.

Antibody assay

a. Antibody assay for ND

The haemagglutination inhibition (HI) assay was carried out in 96-well microplate using two-fold dilution of sera. Washed chicken red blood cells (1%) and four haemagglutinating units of NDV were used. Titres were expressed as $\log_2$ of the highest dilution of serum causing complete inhibition of haemagglutination *(2)*.

b. Antibody assay for RE, ALV-J and CIAV

It was carried out for 30 serum samples using commercial ELISA kits according to the manufacturer's instructions. These kits were evaluated and validated before use:

i. REV antibody test kit (IDEXX Lab., Inc.)
ii. ALV subgroup J. antibody test kit (Synbiotic Co.)
iii. CAV antibody kit (Synbiotics Co.)

Avian Infuenza virus (AIV) isolation

Flu detection kit (synbiotic Co.) was tried to detect AIV infection in tracheal and faecal swabs from affected flock.

Experimental design

Experiment (1)

Investigation of the reason of the proposed vaccine failure in an affected flock (aging 67 days) was carried out through:

a. Testing NDV vaccines used in the affected farm, the MDT, intracerebral pathogenicity index (ICPI) using one-day-old SPF chicks and virus titre was estimated for the LaSota vaccine in SPF embryonated eggs and protective dose fifty (PD_{50}) for inactivated NDV vaccine *(3)*.

b. Checking 25 faecal and tracheal swabs of examined birds for presence of AIV by the antigen capture ELISA (AC-ELISA).

c. Serological monitoring of the affected flock sera for CIAV, NDV, RE and ALV-J antibodies (using ELISA test) and NDV antibodies (using HI test).

Experiment (2)

CIAV infected group (group 1)

Eighty one-day-old SPF chicks were inoculated IM with 0.1of CIAV, vaccinated with HB_1 at 6^{th} day of age and with IBD, intermediate plus type vaccine, at 13 days of age. Birds were separated into 2 subgroups (1 and 2) each consisted of 40 birds. The first subgroup was vaccinated with LaSota and the 40 birds with inactivated NDV at age of 18 days respectively.

IBD vaccine control group (2)

Eighty one-day-old SPF chicks of the same hatch were used as control, divided into two subgroups (4 and 5) and received the same treatment of group (1) respectively except infection with CIAV.

Negative control group (3)

Eighty one-day-old SPF chicks of the same hatch, received the same treatment of group 1, except CIAV and IBD vaccine.

Subgroups Treatment

Subgroup 1: Received CIAV-IBD-Hitchner B1 vaccine and LaSota.

Subgroup 2: Received CIAV-IBD vaccine-Hitchner B1 and inactivated NDV vaccine.

Subgroup 3: Received IBD vaccine-Hitchner B1 and LaSota vaccine.

Subgroup 4: Received IBD vaccine-Hitchner B1 and inactivated NDV vaccine.

Subgroup 5: Received Hitchner B1 and LaSota vaccine.

Subgroup 6: Received Hitchner B1 and inactivated NDV vaccine.

All chickens were maintained in isolation and were examined daily throughout the experimental period. The hematocrit values were determined at two weeks of age, chickens were regarded as anaemic when they had a hematocrit value below 27% ***(4).***

Two weeks post NDV vaccination, 10 birds from each group were weighed, blood samples were taken and sera were separated and assayed for HI antibodies, slaughtered and examined for macroscopical abnormalities. Another 15 birds from each group were challenged with velogenic viscerotropic NDV and the protection rates were estimated during 21 days observation post challenge.

RESULTS

Experiment 1

The evaluation of NDV vaccines used in vaccination of affected flock for pathogenecity and potency revealed that the LaSota vaccine titre was $\geq 10^{7.0}$ EID_{50}/field dose, MDT was about 102.4 hours as shown in Table 1 and the index of neuro-virulence was 0.4 as shown in Table 2. The PD_{50} of the inactivated NDV vaccine was 82/ 0.5 ml. Testing of all examined faecal and tracheal swabs from affected flock failed to demonstrate presence of AIV infection.

The results of NDV-HI antibody assay were depicted in Table 3. The antibody levels between 5 and 9 log_2 with geometric mean titre (GMT) of 111 log_2.

The serological monitoring using ELISA kit were negative for REV and ALV-J antibodies.

The result of CIAV antibody assay as shown in figure 1, levels between 2.76 and 4.23 log_{10}. 20% of tested sera were less than 3 log_{10}, the majority of CIAV ELISA titres ranging from 3 log_{10} to 4 log_{10} representing were 53.3% of 30 serum samples, while positive ELISA antibody levels over 4 log_{10} were 26.7%.

Experiment 2

Clinical and macroscopical findings

The hamatocrit values are shown in Table 4. The chickens in the control groups 2 and 3 showed no anemia in any chicks. While all chickens of CIAV infected group 1 were significantly anemic at 14 days of age.

Clinical observation of 3 groups showed depression, anorexia in the first subgroup with severe respiratory signs and conjunctivitis, these signs were particularly evident 10 days post NDV vaccination. The second subgroup showed depression, dehydration and ruffled feathers. Neither clinical signs nor gross lesions were detected in groups 2 and 3. The mortality rates were higher in first and second subgroups as shown in Table 4. Eight chickens out of 40 were died within 2 weeks post LaSota vaccination in the first subgroup. The second subgroup showed 12.5% mortality. The non-CIAV- infected control groups 2 and 3 only one died. The macroscopic examination of the died birds showed paleness of the carcasses, pale bone marrow, atrophy of thymus and bursa and liver was swollen and yellowish.

Table 4 shows the mean of the body weights in the infected and the control groups, two weeks post NDV vaccination. Retarded growth was noticeable in the CIAV infected two subgroups, the marked retardation was in the first subgroup received LaSota vaccine.

The results of the ND HI antibody assay of the experimentally CIAV-IBD-laSota subgroup 1 showed non-significant ($P \geq 0.05$) decrease than IBD-LaSota subgroup as shown in Table 5. The subgroups 5 and 6 showed higher significant HI responses than subgroups 1 and 3 and 2 and 4, respectively.

Table 5 shows the protection percentages against challenge with velogenic viscerotropic NDV which decreased from 100% in negative subgroups (5 and 6) to 47% in CIAV-IBD vaccine-LaSota subgroup and only 40% in CIAV-IBD vaccine-killed NDV vaccine. Slight decrease in IBDV vaccine subgroup 4 was about 7% than the negative subgroups.

Table 1. Mean Death Time (MDT) of the minimum lethal dose (MLD) of LaSota Vaccine

Virus dilution Log_{10}	No. of deaths at hours post onoculation												Dead/Total Inoculated	Mortality (%)	Mean Death Time (MDT)
	24	32	40	48	56	64	72	80	88	96	104	112			
9	0 *	0	0	0	0	0	1	0	1	0	0	0	2/5	40	MLD = 7.0 MDT = 102.4
8	0	0	0	0	0	0	2	0	0	1	0	0	3/5	60	
7	0	0	0	0	0	0	0	0	0	1	4	0	5/5	100	
6	0	0	0	0	0	0	1	1	1	1	0	0	4/5	80	

$$\text{Mean Death Time (MDT)} = \frac{\text{(Number dead at x hours) X (x hours) + (Number dead at x hours) X (y hours) + etc}}{\text{Total number dead}}$$

$$102.4 = \frac{(1 \times 96) + 4\,(104)}{5}$$

MLD : Minimum lethal dose at dilution 7.0, produced 100 % mortality

Table 2. Result of the ICPI of LaSota vaccine in SPF-day-old chicks

Status	Days								Sum	Factor	Total
	1	2	3	4	5	6	7	8			
Dead	0	0	2	2	3	3	3	3	16	2	32
Signs	0	2	0	1	0	0	0	0	3	1	3
Normal	10	8	8	7	7	7	7	7	61	0	0
									80		35

ICPI = 35/80 = 0.43

Table 3. ND HI antibody in the affected flock

	Distribution of NDV-HI titres (log_2)												
	1<	2	3	4	5	6	7	8	9	10	11	12	GMT
No. of positive sera	0	0	0	0	2	2	3	2	1	0	0	0	111.4

GMT: Geometric Mean Titre

Table 4. Mortality percentage, mean haematocrit values (HVs) and mean body weights (BWs) of infected and control groups

Group	Subgroup No.	HVs *	BWs **	% Mortality ***
1	1	18.1±0.88	292±26	20
	2	17.8±0.56	308±11.6	12.5
2	3	33.2±0.67	368±32.6	0
	4	33.1±0.59	375±21.5	2.5
3	5	31.8±0.55	380±28.2	0
	6	32.7±0.95	369±13.7	0

* Mean haematocrit value at 14 days of age.
** Mean body weight of 10 chickens in grams at age of 32 days.
*** Percentage of mortality over 32 days of age.

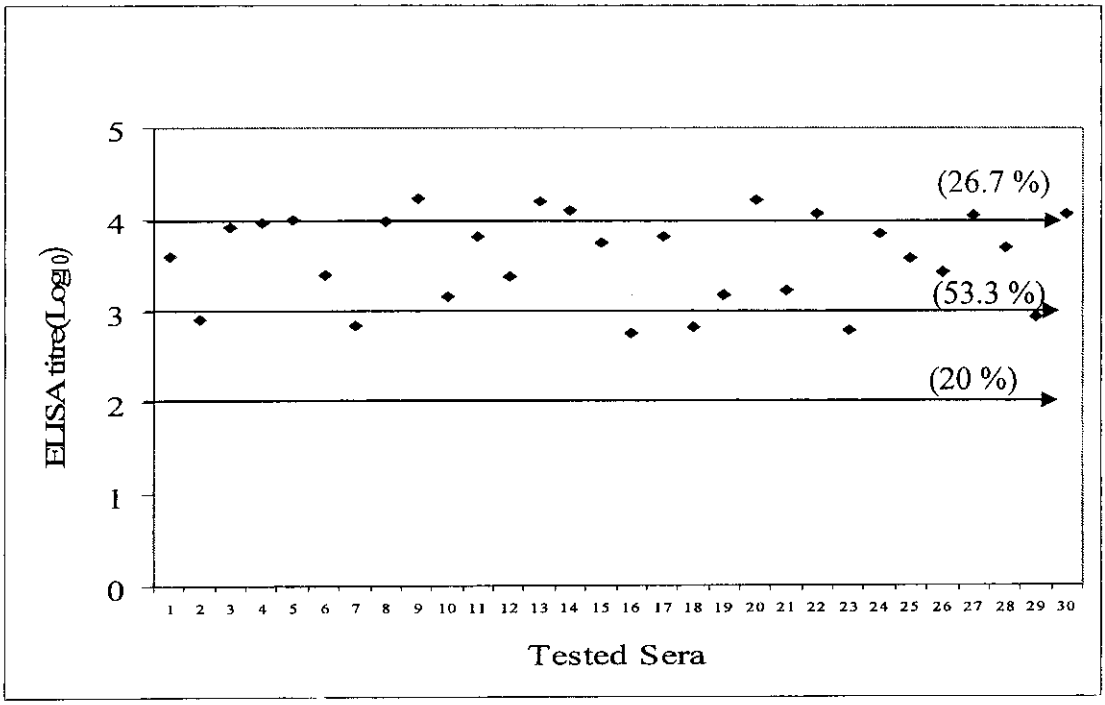

Fig. 1. CIAV ELISA antibody titres of affected flock

Table 5. ND HI responses and protection percentage post challenge in experimental groups

Groups	Subgroups	ND HI titres *										Dead birds**	Protection %
		<1	2	3	4	5	6	7	8	9	GMT		
1	1	0	0	0	2	4	3	1	0	0	37	8/15	46.6
	2	0	0	0	1	3	4	1	1	0	56	9/15	40
2	3	0	0	0	2	3	3	1	1	0	49	1/15	93.3
	4	0	0	0	1	1	5	2	1	0	69	0/15	100
3	5	0	0	0	0	2	5	2	1	0	74	0/15	100
	6	0	0	0	0	0	2	4	3	1	158	0/15	100

* ND HI antibody titre three week post NDV vaccination.
** No. of birds / total challenged birds, 21 days post challenge.
GMT: Geometric Mean Titre.

DISCUSSION

It was demonstrated that the NDV vaccines used in the affected farm complies with the standards of Egyptian regulation for veterinary biologics and complies with *(5)*. The trial to investigate the real cause of immunosuppression were faild for serological finding for RE and ALV-J infection and detection of AIV which may cause severe disease in NDV infected chickens who found that apathogenic AIV H9 isolated in middle east and some Asian countries caused severe NDV infection in chicken*(6)*.

The presence of wide range from 2.76 to 4.23 $\log_{10}$ CIAV-ELISA antibody titres indicative previous CIAV exposure and using an intermediate plus live IBD vaccine in this flock (which widely used in different areas in Egypt for protection against the circulating wild very virulent IBD in Egypt), may be the cause of the problem. The study of case control experiment in SPF chicks supports findings associated with CIAV infection in chickens in the field vaccinated with intermediate plus IBD vaccine, indicated by poor performance, increased respiratory signs, retarded body weight and elevated mortality.The IBDV infection of one-day-old chicks did increase the severity and duration of the disease produced by CIAV and essentially eliminated the resistance commonly confronted in older uncompromised birds*(7,8)*. The older birds can develop CIAV lesions or persistant viremia after infection with infectious bursal disease *(9)*. This supports the hypothesis that CIAV infections plays a role in disease syndromes complicated by other viral, bacterial or fungal infection (*10*). The triple infection of SPF with CIAV, IBD vaccine and LaSota vaccine lead to more depression and respiratory signs than CIAV-IBD and killed vaccine, indicating that the CIAV exacerbated the residual pathogenicity of LaSota vaccine and this agree with previously study *(11)* , which demonstrated pathogenicity of clone-30 in CIAV-infected chickens, also the aggravating effect of CIAV for residual pathogenecity of attenuated Marek's disease virus vaccines CVI-988 /Rispens and 988c/R6 *(12)*. Quadri-infection of CIAV, IBD vaccine, NDV vaccine and virulent NDV lead to mortalities reached 60% than those observed in control groups which did not infected with CIAV. The aggravating effect of CIAV on the pathogenesis of virulent viruses in the field has been demonstrated *(13-16)*.

The non-significant differences between the mean NDV HI titres in the two CIAV infected subgroups and the IBD-vac control groups demonstrates that the humoral immune responses against NDV vaccine is not impaired mainly by the exposure to CIAV. Thus, the evidence suggested that the exerting effect of CIAV on other viruses is mainly caused by impairment of T-cell mediated immune functions *(12)*. The poor protection rates against challenge NDV in CIAV infected groups than IBD vaccinated groups may further support this hypothesis.

The infection of chicken with CIAV in combination with use of IBD-intermediate plus vaccine was associated with exacerbated the diminished immune response to NDV assessed by serology and challenge than NDV vaccinated control. Poor ND antibody titres after vaccination with inactivated ND vaccine in broiler breeder showing positive sera for CIAV *(17)*.

The CIAV has been isolated in virtually all countries with a poultry industry *(18)*. The CIAV has been confirmed in Egypt (*19-22*). In recent years, some outbreaks of ND, IBD and infectious laryngotracheitis occurred in Egypt without the obvious influence of infectious immunosuppression. The results in this report indicate that the role of CIAV should be taken into consideration in diagnosis of diseases in the field, and the active immunization of breeder flocks is very important to limit vertical transmission of the virus and maintain levels of maternal antibodies in progeny to control lateral or horizontal infection.

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الملخص العربى

أثر الإصابة بفيروس أنيميا الطيور والتحصين ضد مرض التهاب البرسا المعدى على كفاءة لقاحات النيوكاسل

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المعمل المركزى للرقابه على المستحضرات الحيويه البيطريه – مركز البحوث الزراعيه

فى هذه الدراسة تم تصميم تجربة باستخدام ثلاث مجموعات كتاكيت خالية من المسببات المرضية عمر يوم واحد – ٨٠ كتكوت- فى كل مجموعة حيث تم احداث عدوى بفيروس أنيميا الطيور عند عمر يوم واحد فى المجموعة الأولى ، ثم تم تحصين الثلاث مجموعات عند عمر ٦ أيام بلقاح هتشنرب١ ،ثم تحصين المجموعتين الأولى والثانية ضد مرض الجمبورو باستخدام لقاح الجمبورو intermediate plus عند عمر ١٠ أيام . أما عند عمر ١٨ يوم فقد تم تحصين ٤٠ كتكوت فقط من كل مجموعة باستخدام لقاح اللاسوتا . وتم تحصين ال ٤٠ الأخرى من كل مجموعة بلقاح النيوكاسل المثبط . وبعد ١٠ أيام من تحصين اللاسوتا (عند عمر ٢٨ يوم) بدأ ظهور أعراض الخمول والأعراض التنفسية مع وجود نسبة نفوق عالية فى المجموعات المصابة بأنيميا الطيور والمحصنة ضد مرض الجمبورو واللاسوتا . وعند عمل اختبار التحدى باستجدام العترة Velogenic Viscerotopic من فيروس NDV قلت نسبة الحماية فى المجموعات المصابة بأنيميا الطيور إلى 40% مقارنة بالمجموعة الثالثة (غير مصابة بأنيميا الطيور ولم تحصن ضد مرض الجمبورو) حيث كانت 100% .