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The Serological Responses in Chicks Vaccinated with Combined or Single Infectious Bursal Disease and Newcastle Disease Vaccines

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Abstract: The antibody (Ab) responses in chicks vaccinated against infectious bursal disease (IBD) and Newcastle disease (ND) using combined and single vaccination regimes were determined. Three vaccination routes namely aerosol, intranasal (I/N) and drinking water (DW) were used. Enzyme-linked immunosorbent assay (ELISA) was employed to measure the Ab responses to the vaccines. Generally, better Ab responses were observed for both vaccines when given in a single form as compared to the combined form except for the IBD when given in DW with non-significant ($P<0.05$) difference and for ND when given in aerosol with significant ($P<0.05$) difference. However, the overall combined vaccination gave better Ab responses than single vaccination. The highest Ab response noted for IBD was when the vaccine applied via the aerosol in a single form whereas the highest Ab response for ND was noted when the vaccine applied via the aerosol in a combined form. In conclusion, the results obtained revealed that better Ab responses for the vaccines were obtained when they administered via the aerosol. It is, therefore, recommended to vaccinate the chicks against IBD and ND via the aerosol with the vaccines in a combined form as the way described in this scientific communication.

Key words: ND, IBD, combined, single vaccination

Introduction

Infectious bursal disease (IBD) is an important immunodepressive disease of chickens caused by a virus belongs to the family *Birnaviridae* (Murphy *et al.*, 1999). Under natural conditions, chickens acquire infections by the virus via the oral route and both humoral and cellular immune responses are compromised (Sharma *et al.*, 2000). Newcastle disease (ND) is a contagious and fatal disease affecting many species of birds caused by a virus belongs to the family *Paramyxoviridae* (Murphy *et al.*, 1999). Transmission of Newcastle disease virus (NDV) between birds is by inhalation (Alexander, 1990). The modes of the disease transmission are correlated with the routes of the vaccine application. This is the basis for application of live vaccines by spray and aerosol generators (Meulemans, 1988). Ingestion of infected feces is also proved as the main method for bird to bird spread of virulent enteric NDV. Similarly, this assumed the application of the vaccines via the drinking water and food (Alexander *et al.*, 1984). Combined vaccination emerged as an important alternative for single mass administration of live virus vaccines and applied in human and veterinary practice with intention of minimizing the cost and efforts normally exerted in vaccination programmes. Favorable results were obtained when combined avian viral vaccines used (Lutticken *et al.*, 1982; Elham *et al.*, 1995). In this study, the applicability and efficacy of combined IBD and ND vaccines were examined.

Materials and Methods

Chicks: A total of two hundred and ten, one-day old chicks of Loghman breed were used in this study. They were obtained from Arab Company for Production and Agricultural Industry (ACPAI) (Khartoum, Sudan). Chicks were divided into groups and reared in special metal cages till the required age. They were either vaccinated with infectious bursal disease (IBD) and Newcastle disease (ND) vaccines in a combined form when they were 10 days old or with IBD vaccine at two-weeks of age and with ND at three weeks of age.

Vaccines: The vaccines used in the study were as follow:

a. IBD vaccine: a live freeze-dried vaccine containing D78 strain of IBDV as an intermediate vaccine. Each dose contains at least 4.0 log10 (104EID50 dose).
b. ND vaccine: freeze-dried live, chick embryo adapted vaccine containing Komarov strain (K) of ND.

Both vaccines are produced and kindly supplied by the Poultry Viral Vaccines Unit, Central Veterinary Research Laboratory (Khartoum, Sudan).

Vaccines Preparation and Administration: For the aerosol method of vaccination, the vaccines were dissolved in

a quantity of 1000 doses per liter. They were sprayed as a coarse spray evenly over the birds at a distance of 30-40 cm. The aerosol generator (Black and Decker, model 8.102, Switzerland) was used with a flow rate of 2.5ml /second and a particle diameter of approximately 33 mm as measured on water sensitive paper.
For the intranasal (I/N) route of vaccination, the vaccines were dissolved in physiological saline solution (usually 30 ml per 1000 doses) and administered by a standardized dropper in which one drop applied intranasally.
For administration of the vaccines in drinking water (DW), They were dissolved in an amount of water that can be consumed by the birds within approximately two hours.

Experimental Design: The chicks were divided into seven groups (30 chicks each). Chicks in the first, second, third, fourth, fifth and sixth groups were vaccinated with the vaccines as aerosol combined, aerosol single, I/N combined, I/N single, DW combined and DW single respectively. Chicks in the seventh group left without vaccination as control.

Blood Sampling: The heart puncture method was employed for blood collection from chicks. One-milliliter disposable syringes were used for this purpose. Collected blood was left over-night at room temperature to clot and then centrifuged at 1000 rpm for 10 minutes. Separated serum samples were stored at -20°C till used.

ELISA for Detection of Ab Responses: The ELISA kit used for detection of Ab responses to IBD and ND was developed by the animal production and health section, the joint FAO/AEA division. NDV or IBDV coated plates were removed from sealed bag and the locations of samples were in the plate were recorded on a template. Then diluted test sera (diluted in phosphate buffer at 1:500) were added into the appropriate wells and the plate was covered with lid and incubated at room temperature (22-27°C) for 30 minutes. The contents of wells were aspirated and the plate was washed four times with the washing buffer (Phosphate buffered saline with Tween20), inverted and tapped firmly on absorbent paper. 100 μ1 of conjugate reagent (Pre-diluted sheep anti-chicken immunoglobulin peroxidase-conjugated) was added to each welland the plate was again covered with the lid and incubated at room temperature for 30 minutes. The plate was washed as above. 100 μ1 of prepared substrate reagent was added to each well and the plate was covered with lid and incubated at room temperature for 15 minutes. 100 μ1 of stop solution was added. The microtitre plate was blanked in the air and the reading was recored by reading spectrophotometrically at 405nm. Positive and negative sera were used as controls as instructed by the manufacturer.

Statistics: The significance between groups of data obtained were determined using the Duncan Multiple Range test.

Results

The mean antibody titres to IBD and ND vaccines using different regimes and routes of vaccination as detected by ELISA are demonstrated in Tables 1 and 2 respectively.

Antibody Responses to IBD: The highest Ab levels noted to IBD when the vaccines administered via aerosol in a single form whereas the least level observed when vaccines given I/N combined. Application of he vaccines in a single form was found to yield better Ab responses to IBD as compared to combined form except for the DW route with non-significant variation ($P < 0.05$). Aerosol route gave better Ab responses followed by I/N and DW.

Table 1: Antibody titres to IBDV using different regimes and routes of vaccination as detected by ELISA

Vaccination regime	Mean titer	CV%
Aerosol combined	5072*[b]	74
Aerosol single	6763[a]	59
Intranasal combined	3849[b]	67
Intranasal single	4921[b]	40
Drinking water combined	4695[b]	52
Drinking water single	4485[b]	76
Control	1917[c]	54
±SE	598.1	

*Mean titre of Abs to IBD in sera collected from chicks vaccinated by IBD vaccine containing the D78 strain of the virus and ND vaccine containing Komorov strain of the virus.
Means with the same letter are not significantly different at $P = 0.05$
CV% = Coefficient of variance
±SE = Standard error

Table 2: Antibody titres to NDV using different regimes and routes of vaccination as detected by ELISA

Vaccination regime	Mean titer	CV%
Aerosol combined	2735*[a]	162
Aerosol single	1080[b]	199
Intranasal combined	866.7[b]	117
Intranasal single	1651[ab]	92
Drinking water combined	505.9[b]	112
Drinking water single	1748[ab]	127
Control	1048[b]	113
±SE	482.9	

*Mean titre of Abs to ND in sera collected from chicks vaccinated by IBD vaccine containing the D78 strain of the virus and ND vaccine containing Komorov strain of the virus.
Means with the same letter are not significantly different at P = 0.05
CV% = Coefficient of variance
±SE = Standard error

Antibody Responses to ND: The highest Ab levels noted to ND when the vaccines administered via aerosol in a combined form whereas the least level observed when vaccines given DW combined. Application of he vaccines in a single form yield better Ab responses to ND as compared to combined form except for the aerosol route with significant variation ($P < 0.05$) compared to single vaccination. Aerosol route gave better Ab responses followed by DW and I/N.

Discussion

In the present study, the effect of application of combined vaccines of infectious bursal disease (IBD) and Newcastle disease (ND) on Ab levels to these viruses in chicks was studied using different strategies of vaccination. They include combined and single vaccine of IBD and ND and routes employed were aerosol, intranasal (I/N) drops and drinking water (DW). Other research workers have done similar investigation on combined vaccines of IBD & ND (Lutticken *et al.*, 1982; Rhee *et al.*, 1987; Zhu-Ji Mei *et al.*, 1997 and Samina *et al.*, 1999). However, the findings obtained in this study were observed different compared to their results. The discrepancy in the results may should be attributed to the fact that in this study live vaccines of IBD and ND were used, contrary to the previous studies where killed IBD vaccine was used. This explains the difference in the result, as live IBD vaccine should result in immunosuppressed response to ND vaccine in chick sera (Elham *et al.*, 1995; Lukert and Saif, 1997 and Aini, 1999). The mechanisms of immunosuppressive activity of IBD in chicks vaccinated with the live virus had previously been documented (Dohms and Saif, 1984; Nakamara *et al.*, 1992 and Fadel, 1994).

In other studies, similar results to ours were obtained that the vaccination of chicks with combined IBD and ND vaccines at 10 days old is superior over other strategies of vaccination (Lutticken *et al.*, 1982). However, they were also able to detect that the level of Abs formed against ND was not protective when protection test was carried out. This indicates that the protection against these viral infections does not only correlate to the levels of Abs formed against the viral antigens but also to the level of cell-mediated immunity (CMI).

In this study, obvious superiority of combined on single vaccination and aerosol over I/N and DW routes was observed using ELISA technique to measure the Ab responses to the vaccines. Comparable results were obtained in other studies when hemagglutination inhibition test (HI) and agar gel immunodiffusion test (AGID) were employed (Tabidi, 2002).

In conclusion, the superiority of combined over single vaccination and aerosol over other routes of vaccination of chicks against IBD and ND is clearly evident. It is, therefore, recommended to vaccinate the chicks with IBD and ND vaccines in a combined form via the aerosol when they are at 10 days old.

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