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Assessment of the Immunogenic Potential of the Intermediate Infectious Bursal Disease Vaccine Virus (D 78) in Broiler Chicks

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Abstract: The immunogenicity of the intermediate infectious bursal disease (IBD) vaccine virus (D- 78), which commonly used in Sudan, was determined in broiler chicks in this study. The vaccine was employed via three routes of application namely aerosol, intranasal and drinking water. Both agar gel precipitation test (AGPT) and an indirect enzyme linked immunosorbent assay (ELISA) were used to detect the levels of antibody (Ab) responses in sera of vaccinated chicks. The results obtained showed that higher ($P>0.05$) levels of Abs were noted when the vaccine administered via aerosol route as compared to the intranasal and drinking water. The variation in the Ab levels among the chicks vaccinated with either the intranasal and drinking water using both tests was not significant (($P< 0.05$). Following challenge of vaccinated chicks, the protection rates noted are correlated to the levels of Abs elicited. In conclusion, to achieve higher and protective immune status in chicks is recommended to apply the intermediate IBDV (D78) vaccine strain in broiler chicks via the aerosol route. AGPT can be used as a rapid qualitative test to determine the vaccine take among the chicks whereas ELISA should be used quantitatively to determine the levels of Ab responses in vaccinated chicks.

Key words: IBDV, antibody, aerosol, intransal, drinking water, AGPT, ELISA

INTRODUCTION

Infectious bursal disease virus (IBDV) primarily affects the bursa of Fabricius in young birds resulting in impaired immunological capabilities[1,2]. The disease is responsible for high mortality in 3 to 4 week-old chicks, but adult birds remained clinically less affected[3]. The control of the disease mainly through proper immunization as well as maintaining a good hygienic environment[4,5]. Many virus strains had been used as vaccines and classified into mild, intermediate and hot vaccines[6]. Intermediate vaccines were proved to be immunogenic without residual pathogenic effects on the vaccinated chicks[7,8].

Agar gel precipitation test (AGPT) has been routinely used to detect antibody response to IBDV. Though the test is economical and simple to perform, precipitin lines are sometimes not detectable and high antigenic mass is required[9]. The enzyme linked immunosorbed assay (ELISA) is more sensitive, specific and reproducible in detecting antibodies against IBD virus as compared to other serologic tests[10,11]. In the present study, the antibody response and protection potentials of the intermediate IBD vaccine (D78) given to broilers chicks through three different, routinely employed, routes was monitored. The efficiency and applicability of either AGPT or ELISA tests to monitor the antibody response to that vaccine was also targeted.

MATERIALS AND METHODS

Chicks: One hundred and twenty chicks were used in this study .They were obtained as one day old from Arab Company For Production and Agricultural Industry (ACPAI) Khartoum, Sudan, and reared in metal cages till the required age of vaccination.

Vaccine: The IBD -D78 intermediate vaccine, was used to vaccinate the chicks. Each dose of the vaccine contains at least 4.0 log10 (10^4EID50). This vaccine was obtained from Detasi Company (Khartoum, Sudan).

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Methods of vaccine application: For administration of the vaccine in drinking water (DW), the vaccine was dissolved in an amount of water which should be consumed by the birds within approximately two hours. When using the aerosol method of vaccination, the vaccine was dissolved in a quantity of water equal to 1000 doses per liter and spread as a coarse spray evenly over the birds at a distant of 30-40 cm. For the intranasal (I/N) route of vaccination, the vaccine was dissolved in physiological saline solution (usually 30ml per 1000 doses) and administrated by means of a standardized dropper by which drop should be applied intranasally.

Vaccination program: The chicks were divided into four groups namely A, B, C and D (30 chicks per group). The chicks in the groups A, B and C were vaccinated with IBD D78 vaccine at 10 days old, via the aerosol, I/N and DW routes respectively while chicks in group D were left without vaccination as a control. After 15 days of vaccination (25 days old), chicks in all groups were bled by heart puncture method and then, blood was collected. Collected blood was left over night at room temperature to clot and then centrifuged at 1000 rpm for 10 minutes. Separated sera were stored at -20°C before tested for Ab level using AGPT and ELISA. Following 16 days of vaccination (26 days old), chicks were challenged using a virulent IBDV.

Agar gel precipitation test (AGPT): This test was essentially carried out as described by Intervet laboratories (Holland). As the test was performed, the test sera were placed in five outer wells of the gel while the sixth well in the periphery of the Petri dish contained the positive control serum. The antigen was placed in the central inner well. The agar was then incubated in a humidified chamber at room temperature for 48 hours. Then it was read against an illuminated chamber using a magnifying glass. The clear precipitin lines were recorded as positive result.

Enzyme linked immunosorbent assay (ELISA): The ELISA kits used was basically developed by the Animal Production and Health Section, Joint FAO/ IAEA Division. The technique of the test was followed as described by Tabidi and co-workers[12] . The diluted test sera (diluted in phosphate buffer at 1:500) were added into the appropriate wells, already coated with IBDV antigens and the plate was incubated at 37°C for 30 minutes .The contents of wells were aspirated and plate was washed four times with washing buffer (PBS Tween 20). 100 µl of conjugate reagent (pre-conjugated diluted sheep anti-chicken immunoglobulin peroxidase) was added to each well and the plate was incubated at 37°C for 30 minutes. The plate was washed as above. 100µl of prepared substrate reagent (OPD) was added to each well and the plate was blanked in the air and the reading was recorded by reading the optical density (OD) spectrophotometrically at 292nm. Positive and negative sera were used as controls as recommended by the manufacturer.

Statistical analysis: The one-way and two-way analysis of variance (ANOVA) was used to determine the significance between groups of data obtained**.**

RESULTS AND DISCUSSIONS

The antibody responses against IBD vaccine (D78 strain) as detected in chicks by AGPT and ELISA are shown in tables 1 and 2 respectively. Using AGPT, a significantly ($p < 0.05$) higher serologic response was found among chicks vaccinated via the aerosol followed by DW and the least response when chicks vaccinated via I/N.

When ELISA technique was employed to measure the antibody levels to the vaccine, it was observed that significantly ($p < 0.05$) higher Ab levels were obtained when aerosol route was used as compared to I/N and DW (Table 2).

Following challenge of vaccinated birds with the virulent IBDV, the protection rates obtained in the groups of birds vaccinated via the aerosol, I/N and DW were 100%, 92% and 88% respectively.

The serologic response to the intermediate vaccine of IDB (D78), administered via three commonly used routes of application, was assessed in the present study. The protective potential of the vaccine and correlation of that protection to the antibody responses measured by AGPT and ELISA tests was also targeted in this study. It is interestingly that the highest response to the vaccine was observed when given via aerosol route. The ability of this route to elicit high levels of antibody responses to avian viruses was previously confirmed by Giambrone and Hathcock[13]. No significant variation in the response of chickens to the virus when administered via either drinking water or intranasal routes was noted. This promotes the vaccine as a good immunizing agent as these sites are not major sites for the virus replication. The virus was proved specifically replicating in the lymphoid tissues especially those of the bursa of Fabricius[2].

The protection rates obtained following challenge of chicks with the virulent virus strain were observed to correlate to the antibody responses induced by them. This confirmed the potential role of antibody in protection against the IBDV infection, the fact that recently confirmed by Hassan[17].

The results obtained in this study also revealed that AGPT is suitable for screening of the output of the vaccine among different flocks of chickens whereas

Table 1: The serological response to the IBD vaccine (D78) in broiler chicks as determined by the AGPT

Method of vaccination	Total No. of sera tested	No. of positive sera (%)	No. of negative sera (%)
Aerosol (A)	23	21 (91.4)	2 (8.6)
Intranasal (B)	23	16 (69.8)	7 (30.4)
Drinking water (C)	23	20 (86.9)	3 (13.0)
Control (D)	23	11 (48.0)	12 (52.0)

Table 2: The mean antibody titres in chicks vaccinated with the intermediate IBDV vaccine strain (D78) as measured by ELISA.

Method of vaccination	Mean titer	CV
Aerosol (A)	6963* a	59%
Intranasal (B)	4921 b	40%
Drinking water (C)	4695 b	52%
Control	1917 c	52%

*Geometric mean of log10 values of OD read at 410-490 nm. (n=10).
CV= Coefficient of Variance

ELISA proved as very much sensitive and rapid for detection and measurement of antibodies against IBDV. This supports the findings of other research workers published previously[14-16].

In conclusion, the intermediate IBD vaccine proved highly immunogenic and protective when administered in chicks via aerosol route and ELISA is a better serologic technique to monitor that potential of the virus.

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