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Comparative Evaluation of Haemagglutination Inhibition Test and Enzyme-linked Immunosorbent Assay for Detection of Antibodies Against Newcastle Disease Vaccine in Broiler Chicks

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Abstract: The antibody (Ab) titres to the intermediate Newcastle disease virus (NDV) vaccine (Komorov strain) in broiler chicks using haemagglutination inhibition (HI) test and an indirect enzyme-linked immunosorbent assay (ELISA) were compared in this study. The tirtres were compared following vaccination of chicks via the aerosol, intranasal and drinking water routes. For all routes of the vaccine administration, higher Ab tritres were detected using ELISA technique than HI test. For both serological assays, the highest Ab titres detected when the vaccine was administered via the aerosol route with significant level ($p< 0.05$) compared to the control group. Non-consistent pattern in the Ab levels between the two tests was observed for intranasal and drinking water routes. As a conclusion, ELISA proved more accurate, sensitive and rapid but less economic than HI test when used for detection of Ab titres against NDV vaccines.

Key words: ND, antibody, HI, ELISA

Introduction

Newcastle disease (ND) is a fatal and highly contagious disease of poultry (Alexander, 1990). It is enzootic in most countries in Africa, Asia and South America, where it continues to cause serious losses despite the vaccination of industrialized poultry (Aldous and Alexander, 2001). The availability of standard sensitive serological test adapted to the conditions in these countries would facilitate diagnosis and accurate monitoring of vaccination programs. Haemagglutination inhibition (HI) test is the most widely used for measurement of antibodies (Abs) against Newcastle disease virus (NDV) (Allan and Gouph, 1984; Brugh *et al.*, 1978). The test is simple to perform but difficult to standardize among laboratories (Beard and Wilkers, 1985). Enzyme-linked immunosorbent assays (ELISAs) have also been employed for the detection of antibodies against NDV (Synder *et al.*, 1983; Miers *et al.*, 1983; Wilson *et al.*, 1984; Mishra *et al.*, 1985; Adair *et al.*, 1989).

Comparative studies between the two fore-mentioned tests to monitor antibody response to NDV in chicken sera (Marquardt *et al.*, 1985; Bozorghmehrifardl and Mayahi, 2000) and other species sera (Charan *et al.*, 1981; Cadman *et al.*, 1997) had also been conducted. In the present, the results of both HI and ELISA tests used to detect the Ab levels against the merogenic strain of NDV (Komorov) in broiler chicken sera are compared.

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 for vaccination.

Vaccine: The freeze-dried, live, chick-embryo adapted vaccine containing Komorov strain (K) of NDV was used. The vaccine was kindly supplied by the Viral Vaccine Unit, Centre of Veterinary Research Laboratories (Khartoum Sudan).

Vaccination programme: The chicks were divided into four groups namely A, B, C and D (30 chicks per group). Chicks in the groups A, B and C were vaccinated with the NDV vaccine, at 10 days old, via the aerosol, intranasal and drinking water routes respectively whereas chicks in group D were left without vaccination as control. After 15 days of vaccination (25 days old), chicks in all groups were bled by heart puncture method and 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 levels using HI and ELISA.

Haemagglutination inhibition test (HI): Firstly, Newcastle disease virus (NDV) antigen used in the test was

Table 1: Average antibody titres against NDV vaccine antigens as detected by HI and ELISA

Method of vaccination	Ab titres detected by HI	Ab titres detected by ELISA
Aerosol	2.00*±0.17	9.36**±1.86
Intranasal	1.40±0.22	3.22±0.96
Drinking water	1.80±0.16	2.70±1.23
Control	1.08±0.24	1.32±1.56

* $\log_2$ antibody titres detected by HI test (geometric mean±s.d.; n=5)
** $\log_{10}$ antibody titres detected by ELISA (geometric mean±s.d.; n=5)

prepared from Komorov strain of the virus essentially as described by Allan and Gouph (1984). The HI test was carried out according to Abdalla *et al.* (1999). Two-fold serial dilutions of serum samples were made with normal saline in micro titre plates. Volumes of 0.05 ml of the NDV antigen were added in each well of the plate. Three rows of wells were left as controls: the first row contained a known NDV antiserum (positive control), the second row contained NDV antigen alone (negative control) and the third row contained normal saline with RBCs (reagent control). The plate was shaken by a titerteck plate shaker and left for 30 minutes at room temperature before the addition of 0.05 ml of chicken RBCs to each well. The plate was then rotated and left till a pattern of HA appeared. Haemagglutination inhibition titres were expressed as the reciprocal of the highest dilution that cause 50% inhibition of agglutination (Allan *et al.*, 1978). The base two logarithmic titre was calculated.

Enzyme-Linked Immunosorbent Assay (ELISA): The ELISA kit used was basically developed by the Animal Production and Health Section, Joint FAO/IAEA Division for detection of bovine antibodies and standardized by Bell *et al.* (1991) to detect Abs against NDV. In recent report, we described the ELISA technique used in the present study (Tabidi *et al.,* 2004). The diluted test sera (diluted in phosphate buffer at 1:500) were added into the appropriate wells, already coated with NDV, and the plate was incubated at 37°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 Tween 20). 100 µ1 of conjugate reagent (Pre-diluted sheep anti-chicken immunoglobulin peroxidase-conjugated) was added to each well, and the plate was again incubated at 37°C for 30 minutes. The plate was washed as above. 100 µ1 of prepared substrate reagent (OPD) was added to each well and the plate was incubated at room temperature for 10 minutes. 100 µ1 of stop solution was added. The micro titre plate was blanked in the air and the reading was recored by reading spectrophotometrically at 492nm. Positive and negative sera were used as controls as instructed by the manufacturer.

Statistical analysis: Duncan Multiple Range Test (DMRT) was used to determine the significance between groups of data obtained.

Results

The antibody titres against NDV vaccine (Komorov strain) as detected by HI and ELISA are summarized in Table 1. Generally, higher Ab levels were noted using ELISA technique compared to those detected when HI was used. For both tests used, the highest Ab titres detected when the vaccine was administered via the aerosol route. These titres are significantly ($p< 0.05$) high as compared to the control group. The intranasal and drinking water routes showed non-consistent pattern between the two tests i.e. drinking water titre is higher for HI and intranasal higher for ELISA.

Discussion

The present study was designed and conducted to compare between HI test and ELISA in detecting the antibody responses to the intermediate Newcastle disease virus (NDV) vaccine. The results obtained revealed that ELISA can detect high levels of Abs to the vaccine virus and considered accurate and sensitive compared to HI test. This support the findings of other research workers published previously (Charan *et al.*, 1981; Marquardt *et al.*, 1985; Cadman *et al.*, 1997). The ELISA kit used was primarily produced by the Joint FAO/IAEA Division (1989) for the detection of bovine antibodies to particular antigens. It was then adapted and established for use with NDV and other avian pathogen using a uniform method as described by Bell *et al.* (1991). The kit was designed to be easily transportable and the reagents are sufficiently stable to withstand ambient temperature. The incubation steps take place at 37°C in order to produce uniform results in widely fluctuating ambient temperatures. The test is therefore, proved more practical, sensitive and rapid for detection of Ab titres against NDV vaccines compared to the HI test. However, HI was confirmed more cheaper than ELISA as no micro plate reader is required in addition to the cost of ELISA kit. Similar finding was also published by Bozorghmehrifardl and Mayahi (2000) who showed that HI test is more economic than ELISA kit used for detection of Ab levels against NDV.

The variations in the figures obtained for both tests in all groups of chickens could be attributed to the inherent characteristics of either the tests that the ELISA can detect all functional types of Abs whereas HI can only

detect the haemagglutinating Abs. The higher Ab levels in chicks vaccinated via the aerosol (compared to the other two routes) was confirmed in recent report (Tabidi *et al*., 2004).

Our study concluded that ELISA technique is more accurate, sensitive and rapid to perform in detecting Abs against NDV vaccine compared to HI test although the later is more economic.

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