ORIGINAL ARTICLE **Mirror of Research in Veterinary Sciences and Animals MRVSA/ Open Access DOAJ**

Measurement of antibody titers against Newcastle disease vaccines by Elisa and hemagglutination inhibition test using different methods of administration in Broiler chicks

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ARTICLE INFO

Received: 03.01.2013

Revised: 07. 01.2013

Accepted: 15.01.2013

Publish online:15. 01.2013

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Abstract

Poultry vaccines were widely applied to prevent and control contagious viral diseases. Antibody response produced by Newcastle diseases(ND) virus vaccine which have been given by different routes of administration in broiler chicks using haemagglutination inhibition (HI)test and an Indirect Enzyme-Linked Immunosorbent Assay (ELISA) was determined in this study. One hundred ninety eight ,one day old ,unsexed Ross breed broiler chicks were used for this purpose. The birds were allocated into 6 equal groups ,one for control and the others were vaccinated at 7th day of age with Hitchner B1 and LaSota at 21st and 35th day old via drinking water with skimmed milk(SM), RO water ,aerosol ,intraocular, and intranasal routes respectively .All groups were vaccinated at 14th of age against Infectious Bursal Disease (IBD) .Ten blood samples have been collected from each group at 1st ,21st ,35th ,and 49th day of age .Serum has been separated and stored at-20 c^{0} until analysis. For all routes of the vaccine administration higher antibody titers were detected using ELISA technique than HI test. For both serological assays, the highest antibody titers detected when the vaccine was administered via drinking water route mixed with(SM) with significant level ($p<0.05$) compared to the control regardless to the age, followed by Ro group and intranasl route respectively. The 4th and 5th groups revealed, more or less, the same results. In conclusion, ELISA proved more accurate, sensitive and rapid, but more expensive than HI test when used for measuring of antibody response against NDV vaccines administered with different routes in broiler chicks.

To cite this article: Muhammad H.K. and Ali A.S.AL-Mayah. (2013). Measurement of antibody titers against Newcastle disease vaccines by Elisa and hemagglutination inhibition test using different methods of administration in Broiler chicks. Mirror of Research in Veterinary Sciences and animals. MRSVA2(1), 8-13. DOI: 10.22428/mrvsa. 2307-8073.2013. 00212.x

Keywords: HI, Elisa, SM, RO, ND.

Introduction

Poultry vaccines are widely applied to prevent and control contagious diseases. Their use in poultry production is aimed at avoiding or minimizing the emergence of clinical disease at farm level, thus increasing production (Marangon and Busani, 2006). Newcastle Disease (ND) is one of the most serious endemic diseases in a wide variety of birds. It always leads to considerable chicken death and economic losses in poultry production (Homhuan and prakongpan, 2007). Newcastle disease (ND) is a fatal and highly contagious disease of poultry (Alexander, 2003). It is enzootic in most countries, 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 condition 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; Burgh *et al.*, 1978). The test is simple to perform but difficult to standardize among laboratories (Beard and Wilkers, 1985). ELISA has also been employed for the detection of Abs against NDV (Synder *et al.*, 1983; Adair *et al.*,1989). Comparative studies between the two for-mentioned tests to monitor antibody response to NDV in chicken and other species sera had also been conducted and the results of both tests especially in broiler chicken sera were compared (Bozorghmehrifard and Mayahi, 2000; Tabidi *et al.*, 2004[a]). Different strategies can be implemented to effectively prevent and control the spread of the disease, and control plans often include the use of vaccination. Vaccines are, in fact, an important component of poultry disease prevention and control worldwide. Vaccines and vaccination programs vary widely depending on several local factors (e.g. type of production, level of biosecurity, local pattern of disease, status of maternal immunity, vaccines available, costs and potential losses) (Marangon and Busani,2006). Route of administration exerts an important role in the bird immunity, after establishing the type of vaccine to be used, the method of vaccine administration must be defined in the vaccination program. There are different vaccination programs for broiler to achieve a reasonable protection against NDV. The objective of the present study was to determine efficacy of field ND vaccination program in establishing solid immunity using different methods of vaccination measured by ELISA and HI tests.

Material and methods

One hundred ninety-eight, one-day old, unsexed Ross breed broiler chicks were randomly allocated into 6 equal groups. All birds were kept in separate pens and fed on a commercial ration *ad labium*. Chicks of 5 groups were vaccinated at 7th day of age with Hitchner B1, and at 21st and 35th laSota vaccine has been used, whereas the 6th group was left as control unvaccinated with NDV vaccines . Birds in all experimental groups were maintained in experimental units under similar management conditions and kept for 50 days. Chicks of all groups were similarly vaccinated against IBD at 14th day of age via drinking water route. All vaccines were supplied by Lohman Animal LHA (Germany). Different methods of administration for NDV vaccines have been used as shown in the experimental design (Table 1) below (Awaad *et al*, 2010). Blood samples were obtained through slaughtering each bird and collected individually in plain tubes. Collected blood

was left slantwise over-night at room temperature to clot and then centrifuged at 1000 rpm for 10 min and serum was harvested and stored at-20c^{0} until analysis. The presence of ND Abs in serum samples was measured using the HI method. The reciprocal of the last serum dilution showing an inhibition of hemagglutination of the 8 hemagglutinating units of the laSota virus vaccine was considered as the HI antibody titer of the serum. Firstly, NDV antigen used in the test prepared from LaSota virus vaccine.

Table1: Shows the experimental Design

Group	Route of NDV vaccines administration
A	Drinking: Tap Water + Skimmed Milk(SM).
B	Drinking: Reverse Osmoses (RO water).
C	Aerosol: Distilled water.
D	Intraocular: Distilled water.
E	Intranasal: Distilled water.
F	Control: Unvaccinated with NDV vaccines.

- All groups (except control) were vaccinated With Hitchner B1 at 7th and with LaSote at 21st and 35th day of age.
- Blood samples (about 2ml) were colleted from each bird (5 birds of each group) at 2wks interval after each administration as well as at the 1st day of age from the control group.

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 titer plates. Volumes of 2.5µl of NDV antigen were added in each well of the plate. Two rows of wells were left as control, the first row contained NDV antigen alone (negative control) and the second row contained normal saline with RBCs (reagent control). The plate was left for 30 min at room temperature before the addition of 2.5 µl of chicken RBCs to each well. The plate was then rotated and left till a pattern of HA appeared. The ELISA technique used in the present study was as described by Tabidi *et al*., (2004**^{b}**). The diluted test sera with phosphate buffer were added into wells already coated with NDV, and the plate was incubated at 37C^{0} for 30 min. The contents of wells were aspirated and the plate was washed four times with washing buffer. A 100µl of conjugate was added to each well, and the plate was again incubated at 37C^{0} for 30 min. The plate was washed and 100µl of substrate was added and incubated at room temperature for 10 min. A100µl of stop solution was added and the reading was recorded by reading spectro-photometrically at 492nm as instructed by the manufacturer. Duncan Multiple Range Test (DMRT) was used to determine the significance between groups of data obtained.

RESULTS AND DISCUSSION

Poultry viral diseases constitute one of the major problems facing the rapidly expanding poultry industries, these diseases cause considerable economic loses, such as ND. For this reason, veterinary authorities rely fairly on vaccination. The ideal vaccination regimen is depending basically on selecting the type of vaccination method (Hafez, 2005).

An effective vaccination plan should result in a general improvement of the health status and the productive performance of the vaccinated population. The efficacy of vaccine administration and the level of immunological response in vaccinated birds can be serologically monitored. Two methods are used to measure antibody titers: the HI and ELISA (Alexander, 2003). Table 2 showed the immune response against NDV vaccines which have been administered via different routes in this study as detected by HI and ELISA. Generally, higher Abs levels were noted using ELISA technique compared to those detected with HI. For both tests used, the highest Ab titers detected when mixed with skimmed milk followed by Ro water. These titers are significantly ($p<0.05$) high as compared to the control unvaccinated group.

Table 2: Shows the average antibody titers against NDV vaccines as detected by HI and ELISA

Group	Method of vaccination	Age / Days					
		21		35		49	
		HI	ELISA	HI	ELISA	HI	ELISA
A	Drinking: Tap Water + SM	4.420^{a} ± 0.23	2330.000^{b} ± 0.42	5.012^{c} ± 0.11	5078.300^{d} ± 0.42	6.000^{e} ± 0.31	6385.700^{f} ± 0.42
B	Drinking: Ro water	3.921^{b} ±0.53	1397.600^{c} ±0.53	4.321^{d} ±0.35	2383.200^{cd} ±0.24	5.013^{f} ±0.91	4135.800^{af} ±0.32
C	Aerosol: Distilled water	3.500^{ab}± 0.81	1200.200^{cb} ± 0.72	3.801^{cd} ± 0.93	2104.300^{e} ± 0.51	4.501^{ef} ± 0.54	3115.300^{cf} ± 0.34
D	Intraocular: Distilled water	2.210^{c} ± 0.91	1150.200^{d} ± 0.81	2.551^{e} ± 0.53	1655.400^{f} ± 0.33	2.758^{cf} ± 0.12	1840.500^{ef} ± 0.19
E	Intraocular: Distilled water	2.310^{c} ± 0.51	1136.500^{d} ± 0.18	2.491^{e} ± 0.72	1442.200^{f} ± 0.31	2.685^{cf} ± 0.63	1708.300^{ef} ± 0.35
F	Control: Unvaccinated	1.100^{cb} ± 0.33	258.000^{cd} ± 0.34	0.995^{ce} ± 0.11	166.000^{cf} ± 0.18	0.758^{g} ± 0.22	135.400^{e} ± 0.22

- Log_2 antibody titers detected by HI test (geometric mean ± sd ,n =5 samples)
- Log_{10} antibody titers detected by ELISA(geometric mean ± sd , n =5 samples)
- HI and ELHSA titer at the 1st day of control group was 4.5 ± 0.23 and 2130.000±0.95 respectively.
- Figures with different superscripts in the vertical and horizontal columns were significantly differed at $p<0.05$.

The intranasal and intraocular routes showed non- consistent pattern between the two tests. Tabidi *et al*.,(2004[b]) found that the Ab titers against NDV vaccine (Komorove strain) at 10 days of life was higher for both tests when the vaccine was administered via the aerosol route. This might be due to the type of vaccine, age of the birds as well as the condition of the drinking water. Alexander *et al* ., (2003) stated that it is important not to use chlorine treated tap water and powdered milk to neutralize the effects of the chlorine is necessary. The HI titer gives an indication of immune status of the bird, a titer of $\log_2^3$ in indicative of protection and a titer of $\log_2^6$ or more suggests a recent infection by the virus. An acceptable Ig G titer measured by ELISA that is correlated to protection should be above 1,000. (Khalifeh *et al*., 2009), whereas Wambura , (2009) stated good protection ,should be 6.5-8.0. Group D and E were failed to reach these levels of protection when measured with HI. This may due to the difficulty in application of vaccine to individual birds. An important comparison for the antibody titer with age of testing (21st ,35th and 49th) showed that the level of antibody production increased ($p<o.05$) significantly in group A in both tests followed by group B and C respectively, whereas group D and E showed no significant difference between each other . It is important to mention that the control group had a highly significant decreased ($p>0.05$) in antibody titer from 1 to 49 day, whereas the vaccinated groups showed an opposite trend as indicated in table 1- These findings were in agreement with those of Ali *et al*., (2004) who stated that a solution containing as little as 500 ppm of egg yolk or powdered skim milk (P.S.M) greatly reduced the activity of both chlorine and quaternary ammonium based disinfectants .Al-Mayah *et al*., 2009) concluded that R.o water is a suitable diluents to be used as drinking in vaccination against ND without the addition of P.S.M. because of its purity . HI tested has been used in those studies. Allan *et al*., (1978);Tabidi *et al*., (2004[b])mentioned that higher Ab levels were noted for both HI and ELISA tests when the vaccine was administered via the aerosol route. The basic principle of this study was to compare between HI test and ELISA in detecting the antibody responses to the ND virus vaccines (Hitchner B1 at 7th day and Lasota at 21st and 35th day of age respectively).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 result is supported by the finding of other research workers published previously (Marguardt *et al*., 1985; Cadman *et al* .,1997; Tabidi *et al*., 2001[a])who stated that the test is proved more practical , sensitive and rapid for detection of Ab titer against NDV vaccines compared to the HI

test. HI test was confirmed cheaper than ELISA as no micro plate reader is required in addition to the cost of ELISA kit. Similar finding was also published by Bozorghmehrifardi and Mayahi (2000), they 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 (Tabidi *et al*., 2004[b]). Khalifeh *et al*., (2009) stated that HI test showed the differences between the antibody level result from different vaccination programs while the ELISA was not. However, ELISA detected maternal antibodies with significant titers in sera of commercial chicks during the first three weeks of age, which could prevent the early attacks in such flock.

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