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Determination of serum antibody titres and immune status of layer flocks against Newcastle Disease virus at Chittagong district of Bangladesh

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ABSTRACT

A study was conducted to assess the level of serum antibody titres and immune status of layer birds against Newcastle Disease virus by Haemagglutination Inhibition (HI) test in different areas of Chittagong district during November to December, 2010. Sixteen layer flocks were selected based on different ages of birds. A total of 235 serum samples were collected and tested at Microbiology laboratory of CVASU. HI test was performed using commercial Newcastle Disease vaccine (Avinew®) as a source of 4HAU virus antigen. The antibody titre (GMT) levels in 18-26 weeks age group were found to be 70.198, followed by 47.551, 34.776, 17.281 and 18.855 in 27-40, 41-57, 58-73 and >73 weeks age groups, respectively. Moreover, 100% specific immunity against ND was found in 18-26, 27-40 and 41-57 weeks age groups of birds, whereas 93.33 and 94.73% specific immunity was found in 58-73 and >73 weeks age groups, respectively. On an average, 97.87% layer birds showed specific immunity and 2.13% showed nonspecific immunity against NDV. We considered HI titre of 1:8 or above as specific immunity and less than 1:8 as non specific immunity. Highest HI titre was found at the age of 18-26 weeks and lowest titre was at 58-73 weeks of age. The lower level of HI titre seemed to be directly related to some important factors relating to vaccination which have been highlighted in this paper.

Key words: Antibody titers, Immune status, HI test, Newcastle disease virus, Layer birds.

INTRODUCTION

Newcastle Disease is a serious and commonly fatal disease of chickens caused by a paramyxovirus. This disease is the most important cause of mortality in chickens [1]. It is one of the most common respiratory diseases of poultry and occurs worldwide. The Newcastle Disease virus is a single stranded, non-segmented, enveloped RNA virus belonging to genus paramyxovirus of the family paramyxoviridae. There are three pathotypes or strains of Newcastle disease virus. The strains are highly virulent (velogenic), intermediate (mesogenic) or avirulent (lentogenic) based on their pathogenicity in chicken [2]**.** All strains of Newcastle Disease virus agglutinate chicken red blood cells in vitro (and sometimes red blood cells from other species). The process is known as haemagglutination and is the basis of the common serological test, the haemagglutination inhibition test, used to detect antibodies to this virus [3]. Newcastle Disease virus has potentials for expanding its host range in nature [4]**.** The transmission of NDV occurs through newly introduced birds, selling or giving away sick birds, exposure to fecal and other excretions from infected birds and contact with contaminated feed, water, equipment, and clothing [5]. In chickens, ND is characterized by lesions in the brain, respiratory tract and gastrointestinal tract. Morbidity rates of nearly 100% and mortality rates as high as 90% have been recorded in susceptible chickens. Neurological symptoms or severe depression are the most obvious clinical signs of ND, and some unvaccinated birds may be found dead with no detected sign of prior illness [6]. Newcastle Disease virus infections of poultry range from inapparent to rapid death, depending upon the pathotype of virus involved [7]**.** Wild and domesticated birds harbour the NDV while showing no detectable clinical signs of the disease [8]. In countries where poultry are kept exclusively in bird proof housing, the ability of the feral birds to invade affected flocks and transfer the disease is minimal, whereas birds kept on open range are more likely to be infected with strains carried by feral birds [9,10].

The present study was undertaken with the following objectives: to determine the serum antibody titres and identify the specific immune response of layer birds against Newcastle Disease virus at some selected areas of Chittagong district of Bangladesh.

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MATERIALS AND METHODS

Study areas, sample size and duration

The study was conducted in four selected areas of Chittagong District namely, Chandgaon, Mohra, Kalorghat, and Sitakunda. All laboratory activities were performed at the Department of Microbiology, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh, from November to December, 2010. A total of 235 samples were collected from layer birds with the age ranging 18-26, 27-40, 41-57, 58-73 and >73 weeks. For collection of blood samples the birds were randomly selected from flocks.

Collection and preservation of samples

From each bird 2 ml of blood was collected aseptically from wing vein, placed at room temperatures for 30 minutes and then kept in chilling temperatures for separation of serum from clot. The separated sera were collected in Eppendorf tubes and stored at -20°C until used for further analysis.

Preparation of 1% chicken RBC suspension

For making 1% chicken RBC suspension, 5ml of blood was taken aseptically from 4 weeks old specific pathogen free chickens with a disposable syringe containing ethylene diamine tetra acetic acid as an anticoagulant. After transferring the blood into centrifuge tube, equal volume of PBS was added and centrifuged at 1000 rpm for 10 minutes. The supernatant was poured off. Again 30 volumes of PBS added with blood cells and centrifugation steps repeated for twice. After washing thrice with PBS, 1% chicken RBC was prepared by adding 1 ml RBC to 99 ml of PBS.

Haemagglutination test and determination of 4HA unit virus for HI test

A 50µl of PBS was dispended into each well of one row of the plastic v-bottomed 96 well plate. Then 50µl of virus suspension (Avinew® vaccine, Advance Animal Science Co. Ltd.) was placed into the first well and made two-fold dilutions of the suspension across the row by transferring 50µl of fluid from one well to the next. Then 50µl fluid was discarded from the last well so that the volume in each well remained the same. A control row was also made by the same procedure only by using PBS instead of virus antigen. Then 50µl of PBS was added to each well including control. Finally, 50µl of 1% Chicken RBCs suspension was added to each well, tilted gently and allowed to stand at room temperature for 40 minutes by covering to stop dehydration. The result was read and recorded that 8^{th} well was the last to show haemagglutination (thin film) which indicated 1 HA unit. Determination of 4HA unit virus antigen from this HA results was done as described by Ilaria and Alexander [11].

Haemagglutination Inhibition (HI) test

HI test was performed according to the procedure of OIE [12]. In brief, serial two fold dilution of field sera (50µl) was made with phosphate buffer saline in 96-wells microtitre plate up to 10^{th} well. Equal volume of 4HA units of ND virus was added into each well up to 11^{th} well. For facilitating the antigen antibody reaction, the plates were kept at room temperature for more than 30 minutes. Then 50µl of 1% (v/v) chicken RBCs suspension was added up to 12^{th} well. The 11^{th} well contained antigen and RBCs as a positive control whereas 12^{th} well contained only RBCs as a negative control. The RBCs were allowed to settle down at room temperature for 40 minutes by slightly agitating the plate. After that the test result was assessed by tilting the plates and appearance of a sharp button because of settling of intact RBCs was recorded as positive. Maximum dilution of each sample was considered as the end point of HI test from which serum antibody titre was determined by reciprocal measurement of the observed result.

RESULTS AND DISCUSSION

Out of 235 serum samples collected randomly from different selected commercial layer farms, 230 were found positive for specific immunity against Newcastle Disease virus. A Newcastle Disease HI titre of $\log 2^3$ (1:8) or above is generally accepted as indicative of specific immunity [13]. HI antibody titre

Table-1: Distribution of layer birds based on serum antibody titers against NDV determined by HI test

Age (weeks)	Number of samples	Antibody titres using HI test										
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	GMT
18-26	60	-	-	-	8	17	13	10	6	5	1	70.198
27-40	42	-	-	4	9	11	5	7	2	4	-	47.551
41-57	50	-	-	13	10	5	7	11	3	1	-	34.776
58-73	45	-	3	14	6	15	4	2	-	-	-	17.281
>73	38	-	2	9	16	3	5	3	-	-	-	18.855

Table-2: Showing state of immune response by testing of serum samples of layer birds against NDV

Age (weeks)	No. of samples	Specific immunity (in no.)	Non-specific immunity (in no.)	Percentage of Specific immunity against NDV
18-26	60	60	-	100.00
27-40	42	42	-	100.00
41-57	50	50	-	100.00
58-73	45	42	3	93.33
>73	38	36	2	94.73
Total	**235**	**230**	**5**	**97.87**

in layers of different age groups varied from 1:4 to 1: 1024 (Table 1). In case of 18-26 weeks age group, HI antibody titre varied from 1:16 to 1:1024 with geometric mean titres (GMTs) of 70.198, but in 27-40 and 41-57 weeks age group, HI antibody titre varied from 1:8 to 1:512 with GMTs of 47.551 and 34.776, respectively. Whereas, HI titre ranges from 1:4 to 1:128 in 58-73 and >73 weeks age group with GMTs of 17.281 and 18.855, respectively. The antibody titre of 58-73 and >73 weeks age group was found to be somewhat lower (1:4) to protect the birds from the Newcastle disease infection and this might have been due to inappropriate methods of vaccine applied, poor vaccine quality, vaccine failure, unsuitable vaccination schedule or vaccination technique, immunosuppressive diseases, and therefore, was unable to protect the chicks from NDV infection. Other possible causes for the vaccine failure in developing countries include: poor manufacturing practices of vaccine standards, lack of adequate storage facilities, application of expired vaccines and inadequate vaccine handling during transportation [14]. Heat stress and water deprivation in birds can also lead to production of steroids, resulting in immunosuppression [15]**.** Birds receiving continuous treatment with chlorampenicol and furazolidone have been shown to have impaired immune response [16] which can lead to frequent attack by various infectious diseases.

The present study revealed that birds of 18-26 weeks age group showed relatively higher serum antibody titres (GMT 70.198) against NDV, followed by 27-40 (GMT 47.551) weeks and 41-57 (GMT 34.776) weeks age groups and showed relatively less susceptibility to clinical infection. The antibody titre was higher since we have expected that through vaccination or previous exposure of infection the level of serum antibody titres should increase at adult age. The wider range of Newcastle Disease virus titres in birds may be due to natural infection which is known to produce higher antibody titres than vaccination [17].

Table 2 indicates that 100% specific immunity was recorded in 18-26, 27-40 and 41-57 weeks age groups; whereas 93.33% and 94.73% specific immunity found observed in 58-73 and >73 weeks age groups, respectively. The average specific immunity against NDV was 97.87%. There were 3 birds out of 45 and 2 birds out of 38 which had nonspecific immunity against NDV. These findings corroborate with those of Numan *et al.* [18] who reported that, 100% of layer chickens were positive for specific immunity against Newcastle disease virus in Pakistan. Our findings also supports the report of Ezeokoli *et al.* [6] who recorded 72% prevalence of antibodies against NDV in free range and 62.9% in traditionally managed backyard flocks in Nigeria. The important consideration that might hinder the development of specific immunity was quality of water offered to the birds and was found questionable due to acid base imbalance. Unsuitable vaccination schedule also leads to the neutralization of maternally derived antibodies and resultantly making the birds more susceptible to the infection.

Overall results appreciated that high antibody titer found in layer birds is due to their long time rearing, long time and booster vaccination schedule as well as previous exposure to infections. The study suggest that, to maintain good farm practices it is very important to vaccinate the birds of the flock at proper time with proper dose and schedules. Regular vaccination should be done and before vaccination HI antibody titer level of birds against Newcastle Disease virus must be monitored. From this study we conclude that, the antibody titer against Newcastle Disease virus in commercial layer flocks of Chittagong District was apparently protective due to the maintaining of proper vaccination schedule and also due to the previous exposure of Newcastle Disease virus.

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