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Detection of Serum Antibody Levels Against Newcastle Disease in Broiler Chickens

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ABSTRACT

A serological survey on the prevalence of antibodies to Newcastle disease (ND) virus was carried out in and around District Faisalabad. A total of 312 serum samples were collected from different commercial broiler farms and slaughter shops. Samples were divided into three groups according to the age i.e. 0-3 weeks (56 samples), 3-5 weeks (164 samples), 5-7 weeks (92 samples). Haemagglutination inhibition (HI) test was performed to determine the serum antibodies against ND virus. Calculated geometric mean titers for groups 0-3, 3-5 and 5-7 weeks of age, were found to be 11.91, 10.01 and 15.85, respectively. The results showed that the level of protection of vaccinated birds was unsatisfactory.

Key Words: Newcastle Disease Virus; Serum Antibodies; Haemagglutination; Haemagglutination inhibition

INTRODUCTION

Poultry keeping is the dominant form of poultry production in the developing world. Infectious diseases are one of the main factors constraining the poultry sector. Newcastle disease virus has potentials for expanding its host range in nature (Brandly, 1950). Many species of domestic, semidomestic and wild birds have been found to be susceptible (Khan, 1968; Arshad *et al*., 1988). Newcastle disease (ND) is the most important cause of mortality in chickens (Nguyen, 1992). The spread of ND in areas is normally either via newly introduced birds, selling or giving away sick and carrier birds. In view of this situation, a survey was carried in and around District Faisalabad, with the objectives of determining the prevalence of antibodies against ND virus at different age groups of broiler birds.

MATERIALS AND METHODS

Collection of serum samples. A total of 312 blood samples were collected from different commercial broiler farms and slaughter shops. For haemagglutination inhibition (HI) test, the blood samples were allowed to clot; sera were separated and frozen at -20^{0}C for later use.

Haemagglutination inhibition (HI) Test. The serum samples were tested for antibodies against NDV, using the standard HI method (Allan and Gough 1974). The antigen used was reconstituted commercial NDV LaSota vaccine (Sindh Poultry vaccine center Karachi).

Washing of RBCs. A total of 5 ml of chicken blood was collected aseptically in a disposable syringe containing 1 mL of sodium citrate (4% solution) as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 min. The plasma and buffy coat was pipetted off. After washing thrice with phosphate buffer saline (PBS), 1% suspension in PBS was made to be used in HI test.

Test procedure. The test was performed as described by Allan and Gough (1974). Briefly, after making two fold serial dilution of test serum upto 10th well, 4 HA unit of Newcastle disease virus was added upto 11th well and kept at 25-30^{0}C for 25-30 minutes. A 1% chicken RBCs suspension was added into each well. The samples showing peculiar central button shaped settling of RBCs were recorded as positive and the maximum dilution of each sample causing haemagglutination inhibition was the end point. The HI titer of each serum sample was expressed as reciprocal of the serum dilution.

RESULTS AND DISCUSSION

Out of 312 samples, 306 were found positive for specific immunity to ND virus with overall positive percentage of 98.07% (Table I). A ND-HI titer of $\log_2$ 3 or above is generally accepted as indicative of specific immunity (Allen & Gough, 1974). Using this criterion in the present study, about 1.92% of the birds showed no serological signs of specific immunity to ND while 98.07% of the total birds showed serological signs of specific immunity. However, majority of the cases had no protective levels of antibodies to ND virus.

In the 56 serum samples of birds (0-3 weeks of age), 50 were found positive for specific immunity with a positive percentage of 89.28% (Table I). The HI antibody titer varied from 1:2 to 1:64 with a GMT of 11.91 (Table II). The results showed that the serum antibody titers were too low to protect the birds from the Newcastle disease infection. Similar results have been described by Awan *et al*. (1994). The low level of antibodies might be due to low levels of maternally derived antibodies which are transmitted from the hen to the chicks through yolk and protect the chicks from harmful effects of NDV in early ages, therefor, rendering the chicks to sub-clinical form of NDV infection.

All serum samples from 164 birds ranging from 3-5 weeks of age were found positive for specific immunity with positive percentage of 100% (Table I). HI antibody titer varied from 1:4 to 1:32 with a geometric mean titer of 10.01 (Table II). The results showed that the serum antibody

titers were too low to protect the birds from the Newcastle disease infection. There are several possible reasons for this low level of protection in birds, such as poor vaccine quality, unsuitable vaccination schedule or vaccination techniques, impaired immune-competence due to immune-suppressive substances in the feed or to immune-suppressive diseases, and therefore, unable to protect the chicks from NDV infection.

Out of 92 serum samples in the birds ranging from 5-7 weeks of age, all were found positive for specific immunity. The positive percentage for this group was 100% (Table I). HI antibody titer varied from 1:8 to 1:128 with GMT of 15.85 (Table II). Birds of this group were having higher antibody levels than the previous two groups and showed relatively decreased susceptibility to clinical infection.

In-spite of vigorous vaccination schedules, ND is still havoc to the poultry industry of Pakistan and a number of outbreaks have been recorded even in vaccinated chicken flocks (Siddique *et al*., 1986). One of the causes for outbreaks in vaccinated chickens might be the introduction of new ND virus strains against which the local birds have no or very low immunity and thus leading to vaccine failure. Vaccination failure may be classified as, (i) Vaccine (type, storage, transportation and handling), (ii) Administration of the vaccine, and (iii) Condition of the bird.

Poor vaccine quality is a common problem in developing countries and could be the result of poor manufacturing standards, lack of adequate storage facilities, application of expired vaccine batches, faulty application and vaccine handling during transportation (Vui *et al*., 2002). Exposure to viricidal agent like phenol or alcohol and improper disinfected syringes might have detrimental effects on virus viability. Birds receiving continuos treatment with chloramphenicol or furazolidone have been shown to have impaired immune response (Tariq, 1999). Heat stress and water deprivation also lead to production of steroids and thus resultantly immunosuppression (Sil *et al*., 2002). Poor nutrition like hypoproteinemia may hurt the immune response (Tariq, 1999). Quality of water which is offered to the birds was also found questionable i.e. most areas supplied with water full of salinity which might hinder the development of specific immunity possibly due to acid-base imbalancement. Another weak point might be the quality of water to dilute the vaccine before application. Unsuitable vaccination schedule also lead to the neutralization of maternally derived antibodies and resultantly making the birds more susceptible to the infection. Since (Awan *et al.*, 1994) low ND-HI antibody prevalence is suggestive of an interepidemic phase or early phase of infection, problems with ND outbreaks in the near future may have to be expected unless the vaccination practice is improved substantially. The wider range of NDV titers in birds may be due to natural infection which is known to produce higher antibody titers than vaccination (Luc *et al*., 1992).

In conclusion, the level of protection of vaccinated birds was found unsatisfactory which must be improved by hyper-immunizing the hens before laying and by adopting good management conditions.

Table I. Serum samples showing specific or non-specific immunity to ND by HI test

Age(weeks)	Total samples	Specific immunity	Non-specific immunity	Specific immunity %
0-3	56	50	6	89.28
3-5	164	164	-	100
5-7	92	92	-	100
Total	312	306	6	98.07

Table II. Distribution of birds on the basis of HI titers

Age (weeks)	No. of Samples	Antibody titers using HI test 1:2	1:4	1:8	1:16	1:32	1:64	1:128	GMT
0-3	56	6	11	5	14	18	2	-	11.91
3-5	164	-	41	32	86	5	-	-	10.01
5-7	92	-	-	28	44	12	6	2	15.85

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