SEROLOGIC STATUS OF NEWCASTLE DISEASE IN BROILERS AND LAYERS IN FAISALABAD AND SURROUNDING DISTRICTS

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ABSTRACT

A serological survey on the prevalence of antibodies to Newcastle disease virus (NDV) was carried out in broilers and layers in Faisalabad and surrounding districts. A total of 803 serum samples were collected form broiler farms, layer farms and slaughter shops. Samples were divided into three age groups for broilers; 0-3 weeks (56 samples), 3-5 weeks (164 samples), 5-7 weeks (92 samples) and also into three age groups for layers; 17-27 weeks (168 samples), 27-37 weeks (219 samples) and 37-47 weeks (104 samples). Haemagglutination Inhibition (HI) test was performed to determine the serum antibodies against NDV. Calculated geometric mean titers for broiler groups of 0-3, 3-5 and 5-7 weeks of age were found to be 11.91, 10.01 and 15.85, respectively. These values for layer groups of 17-27 weeks, 27-37 weeks and 37-47 weeks of age were found to be 134.89, 153.46 and 149.62, respectively. The results showed that the level of protection in vaccinated birds was unsatisfactory in broilers, whereas it was satisfactory in layers.

Key words: Newcastle disease virus, serum antibodies, Haemagglutination, Haemagglutination Inhibition.

INTRODUCTION

Poultry keeping is the dominant form of poultry production in the developing world. In Pakistan, the investment in poultry sector is about one billion US dollars. Broiler meat is the cheapest source of animal protein and egg availability is increasing at the rate of 4% annually. Every family in rural and every 5th family in urban areas is associated with poultry production activities in one way or the other (Sadiq, 2004).

Infectious diseases are one of the main factors constraining the poultry sector. In Pakistan, poultry industry is facing various diseases such as Newcastle disease (ND), Infectious bronchitis (IB), Infectious bursal disease (IBD), Egg drop syndrome (EDS), Hydropericardium syndrome (HPS) and Avian influenza (AI). These diseases are causing high economical losses in terms of high mortality, morbidity, stress, decreased egg productions and hatchabilities all over the world, including Pakistan (Alexander, 2000). Newcastle disease is the most important cause of mortality in chickens (Nguyen, 1992) and many species of domesticated and wild birds have been found susceptible to this disease (Pearson and McCann, 1975; Arshad *et al*., 1988; Wernery *et al*., 1992). The spread of ND in areas is normally via newly introduced birds, selling or giving away sick and carrier birds (Tu *et al*., 1998). In view of this situation a survey was carried out with the objective of determining the prevalence of antibodies against Newcastle disease virus (NDV) at different age groups of commercial broilers, as well as of commercial layers, in Faisalabad and surrounding districts.

MATERIALS AND METHODS

Collection of samples

The study was carried out in Faisalabad and surrounding districts of the Province of Punjab, Pakistan from March to August, 2004. A total of 803 blood samples were collected from different commercial broiler farms, commercial layer 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 until later use.

Haemagglutination Inhibition test

The serum samples were tested to determine the 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). For this purpose, 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 minutes and the plasma and buffy coat was pipetted off. After washing

thrice with phosphate buffer saline (PBS), 1% suspension in PBS was used in HI test.

The test was performed as described by Allan and Gough (1974). Briefly, after making two fold serial dilution of test serum upto 10^{th} well, 4 HA unit of Newcastle disease virus was added upto 11^{th} well and kept at 25-30^{o}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 considered as the end point. The HI titer of each serum sample was expressed as reciprocal of the serum dilution.

RESULTS AND DISCUSSION

A total of 803 serum samples were collected from different commercial layer farms, commercial broiler farms and slaughter shops and were subjected to HI test. Out of these, 306 were found positive for specific immunity to ND virus with overall positive percentage of 98.07 in broilers (Table 1), whereas all samples from layers were found positive for specific immunity to ND virus (Table 3).

A ND-HI titer of $\log 2^3$ (i.e. 1:8) and above is generally accepted as indicative of specific immunity (Allan and Gough, 1974). Using this criterion in the present study, 1.93% of the commercial broilers showed no serological evidence of specific immunity to NDV, while 98.07% of the total commercial broilers showed serological evidence of specific immunity. All commercial layers showed serological evidence of specific immunity to NDV. However, majority of the commercial broilers had no protective levels of antibodies to NDV.

Serological status

In commercial broilers, different age groups were split up to elaborate immune response. In 0-3 weeks of age group, out of 56 serum samples, 50 were found positive for specific immunity with a positive percentage of 89.28 (Table 1). The HI antibody titer varied from 1:2 to 1:64 (their separate percentages were 10.71, 19.64, 8.93, 25.0, 32.14 and 3.57, respectively) with a geometric mean titer (GMT) of 11.91 (Table 2). These results show that the serum antibody titers were too low to protect the birds from the NDV 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, therefore, rendering the chicks susceptible to sub-clinical form of NDV infection.

Out of 164 serum samples of broilers ranging from 3-5 weeks of age, all were found positive for specific immunity with positive percentage of 100 (Table 1). The HI antibody titer varied from 1:4 to 1:32 (their separate percentages were 25.0, 19.51, 52.44 and 3.05 respectively) with a GMT of 10.01 (Table 2). This shows 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 immunosuppressive substances in the feed or to immunosuppressive diseases, and therefore, unable to protect the chicks from NDV infection.

Out of 92 serum samples in the broilers ranging from 5-7 weeks of age, all were found positive for specific immunity. The positive percentage for this group was 100 (Table 1). The HI antibody titer varied from 1:8 to 1:128 (their separate percentages were 30.43, 47.82, 13.04, 6.52 and 2.17, respectively) with a GMT of 15.85 (Table 2). Birds of this group showed higher antibody levels than the previous two groups and showed relatively decreased susceptibility to clinical infection.

Inspite of vigorous vaccination schedules, ND is still a 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, leading to vaccine failure.

Other factors like poor vaccine quality is a common problem in developing countries and can 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). Heat stress and water deprivation also lead to production of steroids and thus resultantly immunosuppression (Sil *et al*., 2002). Quality of water which is offered to the birds was also found questionable which might hinder the development of specific immunity possibly 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. Since low ND-HI antibody prevalence is suggestive of an interepidemic phase or early phase of infection (Awan *et al*., 1994), 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 commercial layers, all age groups were found positive for protective immunity with a positive percentage of 100 (Table 3). The HI antibody titer for 17-27 weeks age group varied from 1:32 to 1:512 (their separate percentages were 7.14, 20.83, 37.50, 22.62 and 11.90, respectively) with a GMT of 134.89. Antibody titer for 27-37 weeks age layers varied from 1:128 to 1:512 (their separate percentages were 79.45, 12.33 and 8.22, respectively) with a GMT of 153.46. The HI antibody titer for 37-47 weeks age group varied from 1:128 to 1:512 (their separate percentages were 80.76, 13.46 and 5.77, respectively) with a GMT of 149.62 (Table 4).

In conclusion, the level of protection of commercial broilers was found unsatisfactory which must be improved by hyper-immunizing the hens before laying and by adopting good managemental conditions, whereas, the level of protection in commercial layers was found satisfactory.

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Table 1: Serum samples of broiler chicks showing immune response to NDV using Haemagglutination Inhibition test

Age (weeks)	No. of samples	Specific immunity	Non-specific immunity	Specific immunity (%)
0-3	56	50	6	89.28
3-5	164	164	-	100.00
5-7	92	92	-	100.00
Total	312	306	6	98.07

Table 2: Distribution of broiler birds on the basis of Haemagglutination Inhibition titers obtained against NDV

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

Table 3: Serum samples of layer chicks showing immune response to NDV using Haemagglutination Inhibition test

Age (weeks)	No. of samples	Specific immunity	Non-specific immunity	Specific immunity (%)
17-27	168	168	-	100.00
27-37	219	219	-	100.00
37-47	104	104	-	100.00
Total	491	491	-	100.00

Table 4: Distribution of layer birds on the basis of Haemagglutination Inhibition titers obtained against NDV

Age (week)	No. of samples	Antibody titers using HI test									
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	GMT
17-27	168	-	-	-	-	12	35	63	38	20	134.89
27-37	219	-	-	-	-	-	-	174	27	18	153.46
37-47	104	-	-	-	-	-	-	84	14	6	149.62

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