Pak. J. Agri. Sci., Vol. 50(2), 279-288; 2013
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

USE OF HYPERIMMUNE SERUM FOR PASSIVE IMMUNIZATION OF CHICKS EXPERIMENTALLY INFECTED WITH NEWCASTLE DISEASE VIRUS

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Velogenic strains of Newcastle disease virus (NDV) were isolated from field outbreaks. ELD_{50} of the VNDV was determined at $10^{-5.33}$. La Sota strain and Incomplete Freund's Adjuvant (IFA) were inoculated in adult chicks to raise hyper immune serum. The serum was purified and used to reduce the morbidity and mortality in experimentally infected broiler chicks with Newcastle disease virus (NDV). 120 day-old broiler chicks were divided into four groups i.e. A, B, C and D. At 14th day of age, the chicks were infected with velogenic field isolate of Newcastle disease virus (ELD_{50} = $10^{-5.33}$/0.1mL) and two hours post infection the birds were passively immunized with HIS @ 2mL, 3mL and 6mL, in group B, C and D, respectively, while group A was kept as control. Chicks received 2mL HIS exhibited severe respiratory and enteric signs of Newcastle disease, while chicks received 3mL and 6mL HIS were anorexic in early days and on 10th day post inoculation (DPI) they were recovered. Gross lesions in infected birds were hemorrhages in trachea, proventriculus and intestine, and congestion in infected lungs. Histo-pathological examination showed epithelial sloughing, congestion of blood vessels in group A, mild tracheitis and alveoli of lungs filled with exudates along with lymphocytes in group B, mild lymphocytic infiltration in group C, while trachea and lungs showed no significant change in group D. Higher range of GMT values were observed in group D while low range of GMT values were observed in group A. It was concluded that HIS against NDV can be used to decrease the morbidity and mortality rate in experimentally infected birds.

Keywords: Newcastle disease, hyperimmune serum, passive immunization, morbidity, mortality, clinical signs, gross leisions, histopathology

INTRODUCTION

Newcastle disease is a highly contagious viral disease of poultry and has a devastating effect on economical poultry production (Alexander, 1980; Hafez, 2011; Chukwudi *et al*., 2012; Poorbaghi *et al*., 2012). NDV, belonging to the family *Paramyxoviridae*, has a vide host range and is reported in chicken, pigeons, turkeys, pheasants, dove, partridges, gees, starling and other free flying birds (Vindevogel *et al.,* 1982). ND is responsible for high morbidity and mortality in susceptible birds of all age groups (Calnek, 1991; Yan *et al*., 2011;Siddique *et al*., 2012). NDV is a negative stranded RNA, *Rubula* virus containing six Nucleo-proteins (NP), Phosphoproteins (P), Matrix (M) protein, Fusion (F) protein, Hemagglutinin (H), Nuraminidase (N) and Large (L) proteins. Velogenic strain of ND virus may cause 100% mortality while mesogenic strain causes moderate disease and reduction in egg production (Alexander, 1997). The outbreaks of ND have been reported in vaccinated flocks leading to economic losses (Alexander, 1989), which may be due to failure of achievement of certain levels of antibodies (Abs) against NDV. The impaired production of Abs against NDV may be due to deficiency of vitamins, trace elements, amino acids and minerals in feed. In the vaccinated birds, protection is produced by Abs produced against various components of NDV. Inactivated vaccines produce neutralizing Abs that may not be sufficient to protect against virus. During outbreak of ND, various therapeutic measures are adopted to control mortality like antiviral peptide (Rajik *et al.,* 2009) and use of specific Abs against NDV (Reylond and Maraqa, 2000; Umino *et al.*, 1990).

As the imported antiserums and monoclonal antibodies against ND are very expensive, therefore present study was planned to produce antiserum against ND in chicks and to observe morbidity, mortality, clinical signs, symptoms and gross and histopathological lesions of ND in passively immunized chicks using locally prepared hyper immune serum in chicks.

MATERIALS AND METHODS

This study was conducted at the Department of Veterinary

Pathology, University of Agriculture, Faisalabad in 2010.

A) Isolation and Identification of patho-types of ND Virus: Morbid organs (lungs, trachea and spleen) were collected from suspected field outbreaks of Newcastle disease. Tissues were homogenized in normal saline (1:10) and gentamycin was added at the rate of 1mg/ml (Senne, 1989). The homogenate was centrifuged at 1000×g for 20 minutes at 4ºC and supernatant was stored in aliquots at –20ºC until further processing. Suspected material was inoculated in 9-11 days-old embryonated eggs through chrioallointic route (Hitchner, 1980) Allantoic fluid of dead embryos was harvested and tested for presence of virus by to spot agglutination test, haemagglutination test (Allan *et al.,* 1978) and confirmed by hemagglutination inhibition (MAAF, 1984).

The confirmed isolates were further processed for pathogenicity test based on Mean Death Time the viral isolates were characterized as Lentogenic,Mesogenic,and Velogenic (Alexander, 1989).

The 50% Embryo Lethal Dose of velogenicstarins of NDV was determined by the method of Reed Munch (Villegas and Purchase, 1989). Different dilutions of velogenic isolate of NDV (i.e. 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were used for the determination of ELD_{50}.

B) Preparation of hyper immune serum: 15 adult healthy broiler chicks were used for the production of HIS against NDV according to method described by Iqbal *et al.* (2003). All the birds were housed individually in separate cages and were offered feed and water *ad libitum*,and standard temperature. The blood and fecal samples of all the birds were examined before experimental inoculation and the birds having any kind of parasitic infestation were excluded. Serum from all the birds was collected and observed for haemagglutinating activity after inactivation at 56°C for 30 minutes. 5 out of 15 birds were kept as control, while remaining 10 were injected with NDV (La Sota strain + Incomplete Freund's Adjuvant) intramuscularly according to schedule described in Table 1. Blood samples from the birds were taken periodically to observe their antibody titre against NDV by HI test. The chicks were bled after having highest antibody titer and blood was collected aseptically in sterilized centrifuge tubes without anticoagulant. Serum was separated from the clotted blood and purified.

Table 1. Inoculation schedule.

Injection (Day)	Inoculam type	Quantity of Inoculam (mL)
0	NDV (La Sota)	0.5
14	NDV (La Sota)	0.5
21	NDV (La Sota)	0.5
28	NDV (La Sota) + IFA	0.2
42	NDV (La Sota) + IFA	0.2

(IFA = Incomplete Freund's Adjuvant)

The purification / fractionation of Serum was performed. The major protein component of the serum (globulins) was separated by precipitating with Ammonium Sulphate [$(NH_4)_2$ SO_4] according to the prescribed method (Villegas, 1986) and stored at –20°C until further processing.

C) Inoculation of HIS in Experimental Chick: 120 day-old broiler chicks were divided into four group viz., A, B, C and D, with each group having 30 birds. 0.1mL of Velogenic strain of NDV (ELD_{50} = $10^{-5.33}$/0.1mL) was inoculated subcutaneously to all the groups at age of 15 days, and two hours post infection the group B, C and D were injected HIS @ 2mL, 3mL and 6mL (having antibody titer of 1:512) intra-muscularly (i.m), respectively, while group A was un-inoculated control.

Antemortem and postmortem examination: Clinical signs and symptoms along with mortality rate and morbidity rate were recorded up to 2 week post infection with NDV. Visceral organs like lung, trachea, proventriculus and intestine of dead and slaughtered birds were examined for gross lesions.

Histopathological studies: After infecting with ND virus in experimental chicks and inoculating with HIS the visceral organs like, lung, trachea, proventriculus and intestine of dead and slaughtered birds were processed for histopathological examinations according to the procedure (Bancroft and Gamble, 2007).

Humoral immune response of experimental groups: Serum was collected from 7 chicks of all the groups at 4^{th}, 8^{th}, 12^{th} and 14^{th} DPI. Antibody titer against NDV was determined through Haemagglutination Inhibition test and Geometric Mean Titer was calculated.

RESULTS

Haemagglutination and HI test: The chorio-allantoic fluid of three viral isolates viz; A, B and C having positive spot agglutination test had heagagglutinating titer of 1:64, 1:256 and 1:8 respectively. These isolates were inhibited by specific hyper immune sera against Newcastle disease virus.

A)Mean Death Time of chicken embryos: MDT of isolate A ranged from 54 to 60 hours with dilutions of 10^{-4} and 10^{-5}. These isolates were characterized as velogenic. MDT of isolate B ranged from 66 to 72 hours with dilutions of 10^{-4} and 10^{-5} while at this same dilutions MDT of isolate C ranged from 84 to 90 hours characterizing both as mesogenic strains. All the viral isolates were inhibited by known hyper immune sera against NDV.

Embryo Lethal Dose$_{50}$: The calculated ELD_{50} of velogenic strain (Isolate A) was $10^{-5.33}$/0.1mL of virus.

B) Production of hyperimmune serum against NDV in chicks: No antibody titer against the antigen was found at 0 and 7^{th} days post inoculation. Then it gradually increased with the passage of time to the titer of 1:64, 1:128, 1:256 at 21, 28 and 35 days respectively. At day 42, the titer reached at 1:512 and remained constant up to 49^{th} DPI.

C) Observations of passive immunization in experimental Groups:

Mortality and morbidity rate: In Group A (control group) 30 birds became diseased and 9 birds died, resulting in morbidity and mortality rate of 100% and 33.3%, respectively. In group B 15 birds became diseased and 7 birds died, having morbidity and mortality rate of 50% and 23%, respectively. In group C 9 birds became diseased and 3 birds died, resulting morbidity and mortality rate of 33.3% and 10% respectively. In group D three birds became sick and one bird died, having morbidity and mortality rate of 10% and 3%, respectively at 15th DPI (Table 2).

Table 2. Mortality and mortality rate in groups passively immunized with different doses of HIS.

Experimental Group	Morbidity		Mortality	
	Birds affected	Morbidity rate (%)	No. of birds died	Mortality rate (%)
A	30	100.0	9	33.3
B	15	50.0	7	23.0
C	9	33.3	3	10.0
D	3	10.0	1	3.0

Clinical signs: In group A chicks were infected with NDV and not inoculated with HIS. No clinical signs appeared till 2nd DPI in the control group. On 4th DPI, 5 chicks became off feed and depressed and their feed intake also decreased. Decreased feed intake was observed in 18 chicks up to two weeks post-inoculation. On the 6th DPI, 7 chicks were suffering from greenish diarrhea which increased in 11 birds persisted up to two weeks. On 8th DPI, nasal discharge started along with difficult breathing which spread rapidly and it was recorded in 10 chicks while 17 birds suffered from difficult breathing on 12th and 14th DPI. Nervous signs appeared on 14th DPI, blindness and torticulus was also recorded in 8 and 3 birds, respectively. Rests of the birds were suffering from diarrhea and nasal discharge.

In group B chicks were infected with NDV and inoculated with 2mL HIS. No clinical signs appeared till 4th DPI. On 6th and 14th DPI, 5 to 10 chicks were anorexic. On the 10th DPI, 7 chicks were suffering from greenish diarrhea which increased up to 10 birds on 14 DPI. On 12th DPI, difficult breathing was recorded in 10 chicks. On 14th DPI same clinical signs were observed in 13 birds.

In group C chicks were infected with NDV and inoculated with 3mL HIS. No clinical signs appeared till 6th DPI. On 8th DPI, 5 chicks were anorexic. From 10th to 14th DPI anorexia was observed in 9 chicks. On 10th DPI, 5 chicks started suffering from greenish diarrhea. On 12th DPI, difficult breathing was recorded in 8 chicks. On 12th DPI 11 birds showed signs of difficult breathing.

In group D chicks were infected with NDV and inoculated with 6mL HIS. No clinical signs appeared till 6th DPI. From 8th DPI, birds became off feed and depressed up to 14th DPI. After that the birds recovered from infection and started taking feed normally. The appearance of clinical signs in birds in different groups after inoculation of different doses of HIS is depicted in Table 3.

Gross lesions: In group A, on 2nd DPI seven birds were slaughtered and observed for gross lesions. No significant lesions were found upto 4th DPI. On 6th DPI as greenish diarrhea appeared, hemorrhage in intestine and proventriculus was observed. At 8th and 10th DPI mild hemorrhages in trachea and air sacculitis were seen. On 14th DPI congestion and oedema was observed in the brain of

Table 3. Clinical signs of ND in groups passively immunized with different doses of HIS.

Experimental Group	Type of Clinical Signs	Days Post Inoculation of HIS						
		2nd	4th	6th	8th	10th	12th	14th
A	Anorexia	-	5 (16%)	7 (23%)	9 (30%)	10 (33%)	13 (43%)	18 (60%)
	Greenish Diarrhea	-	-	7 (23%)	9 (30%)	10 (33%)	11 (36%)	11 (36%)
	Difficult Breathing	-	-	-	10 (33%)	7 (23%)	17 (56%)	17 (56%)
	Nervous Signs	-	-	-	-	-	-	11 (36%)
B	Anorexia	-	-	5 (16%)	5 (16%)	7 (23%)	8 (26%)	10 (33%)
	Greenish Diarrhea	-	-	-	-	7 (23%)	8 (26%)	10 (33%)
	Difficult Breathing	-	-	-	-	-	10 (33%)	13 (43%)
	Nervous Signs	-	-	-	-	-	-	-
C	Anorexia	-	-	-	5 (16%)	6 (20%)	7 (23%)	9 (30%)
	Greenish Diarrhea	-	-	-	-	5 (16%)	7 (23%)	9 (30%)
	Difficult Breathing	-	-	-	-	-	8 (26%)	11 (36%)
	Nervous Signs	-	-	-	-	-	-	-
D	Anorexia	-	-	-	3 (10%)	3 (10%)	4 (13%)	6 (20%)
	Greenish Diarrhea	-	-	-	-	-	-	-
	Difficult Breathing	-	-	-	-	-	-	-
	Nervous Signs	-	-	-	-	-	-	-

cockerels showing nervous signs. Mild conjunctivitis was observed in 8 cockerels infected with NDV. In group B, on 2nd, 4th and 8th DPI, no significant lesions were found. From 10th DPI hemorrhages in trachea, proventriculus and intestine were observed. In group C no lesions were observed up till 10th DPI. On 12th DPI mild enteritis and mild sacculitis was observed in 7 cockerels. In group D no lesions were observed throughout the trial.

Histopathological studies: Different pathological changes were observed in different organs at 5th DPI. In Group A, epithelial sloughing, congestion of blood vessels of mucosa and infiltration of inflammatory cells in lamina propria of intestine, catarrhal tracheitis, congestion and necrosis in mucosa of trachea (Fig. 1) and alveoli of lungs filled with exudate along with infiltration of lymphocytes, emphysema and necrosis were observed (Fig. 2).

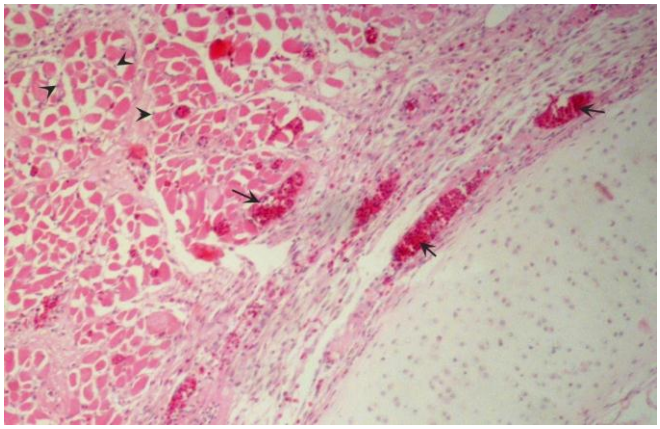

Figure 1. Congestion (→) and necrosis (►) in Trachea in Group-A at 5th DPI.

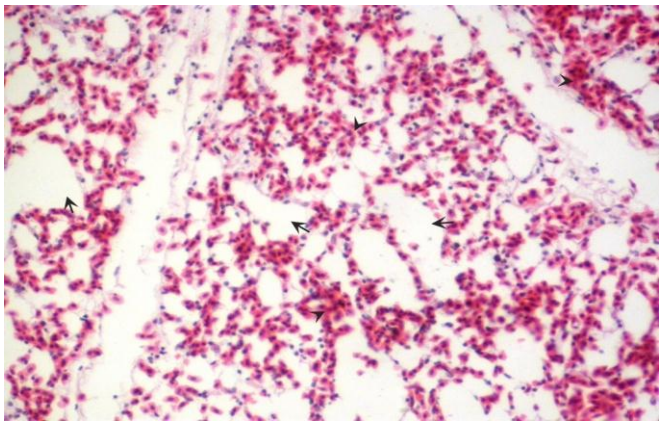

Figure 2. Emphysema (→) and congestion (►) in lungs in Group-A at 5th DPI.

In Group B, mild tracheitis and focal hemorrhages in trachea, alveoli of lungs filled with inflammatory exudates, epithelial sloughing, congestion and infiltration of inflammatory cells in lamina propria of intestine, hemorrhages and necrosis in spleen were observed (Fig. 3).

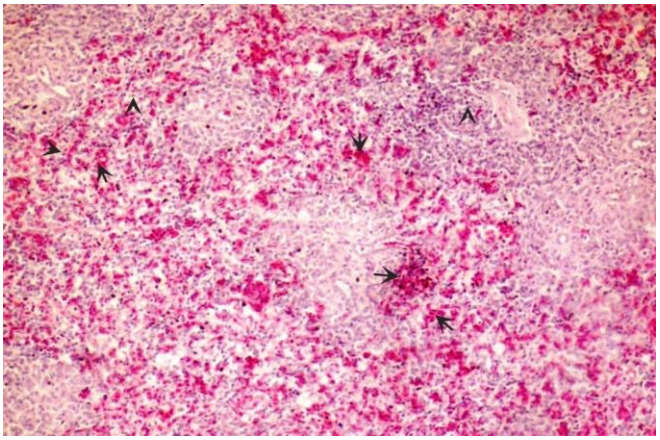

Figure 3. Hemorrhages (→) and necrosis (►) in spleen in Group-B at 5th DPI.

In Group C, epithelial sloughing, congested blood vessels of mucosa of intestine, mild trachitis, hemorrhages and lymphocytic infiltration in mucosa of trachea, alveoli filled with exudates, congestion and mild degree of necrosis were observed (Fig. 4).

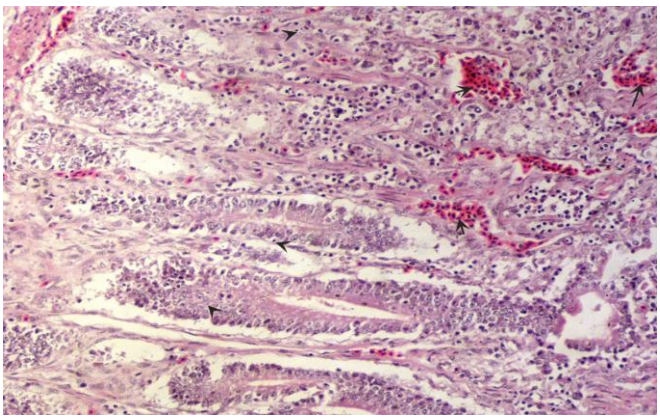

Figure 4. Congestion (→) and mild degree of necrosis (►) in intestine in Group-C at 5th DPI.

In Group D, no significant changes was seen in trachea and lungs, and no epithelial sloughing but only mild infiltration of inflammatory cells in lamina propria of intestine was observed (Fig. 5).

Humoral Immune response of Experimental groups: GMT values of group A, B, C and D ranged from 22.6 to 73.3, 207 to 111.4, 256 to 168.9 and 362.5 to 274.1, respectively, from 4th to 14th day (Table 4).

Table 4. GMT of experimental groups infected with NDV and inoculated with HIS.

Experimental Group	Days Post Inoculation of HIS			
	4th	8th	12th	14th
A	73.3	55.7	34.3	22.6
B	207.0	147.0	137.0	111.4
C	256.0	207.0	207.0	168.9
D	362.5	337.8	294.1	274.1

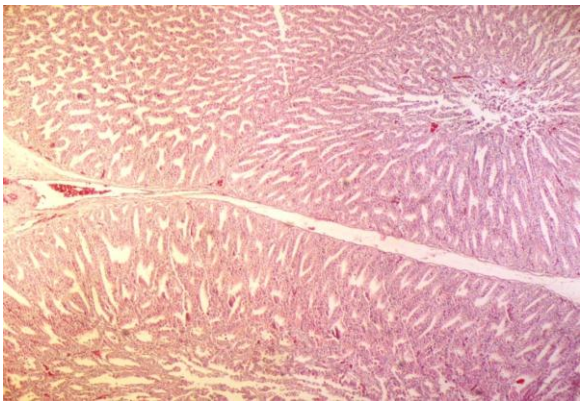

Figure 5. HIS treated birds showing no significant change in Group-D at 5th DPI.

DISCUSSION

Newcastle disease is regarded as very significant pathogen for chicks. The virus is able to infect large number of avian species. The workers in the field of poultry pathology agree that at some occasions there is a definite need for an agent capable of conferring an immediate though not necessarily a lasting immunity to birds exposed to ND virus. In case of valuable and breeder stock the use of antiserum confers passive immunity to chicks which had previously been exposed to ND (Moynihan *et al.*, 1954).

In our study the Newcastle disease virus was isolated from morbid organs (lungs, trachea and spleen) from suspected field outbreaks of Newcastle disease. Hussain *et al.* (1988) also isolated velogenic strain of newcastle disease from outbreak on local poultry farm and propagated the virus in 9-day old embryonated eggs through allantoic route and harvested the allantoic fluid after 48 hours and tested for spot haemagglutinating activity.

In our study the Allantoic fluid was harvested with viral isolates and subjected to spot agglutination test, HA test (Allan *et al.*, 1978) and confirmed by HI test (MAAF, 1984). These tests are specific for the confirmation of ND virus and were also used by Azam *et al.* (1984) and Murakawa *et al.* (2000). Serological tests like HI allow rapid identification of most of samples and are reliable, sensitive, specific and more accurate methods to detect the viruses for the confirmatory diagnosis of disease (Hasan *et al.*, 2010)

In our studyHA test was performed for the isolates A, B and C which gave positive spot agglutination. HA titer of isolates A, B and C was 1:64, 1:256 and 1:8 respectively. All the viral isolates were confirmed by HI test. These isolates were inhibited by specific hyperimmune serum raised against NDV.

In our study a widely accepted protocol for calssifying field isolates based on MDT was used. According to this criteria lento-genic strains possesss MDT > 90 hours, (Samuel *et al.*, 1979; Azam *et al.*, 1984; Shirai *et al.*, 1986; Khalafalla, 1994; King, 1996) mesogenic strains possess MDT 60-90 h, (Afzal, 1990; Reddy *et al.*, 1993; King, 1996; Parimal *et al.*, 1997; Shirai *et al.*, 1986) and velogenic strains possess MDT < 60 hrs (Namita *et al.*, 1995; Parimal *et al.*, 1997). Other scientists had also used MDT to check the pathogenicity of virus (Khadzhiev, 1984; Khalafalla, 1994; Parimal *et al.*, 1997; Roy *et al.*, 1998; Manin *et al.*, 2002).

The production of antibodies is a complex biological phenomenon. It is not always possible to follow any recommendations and guidelines outlined in literature, as procedures and protocols have to be modified depending on the antigen. For some purposes, a single injection may be sufficient but in general higher antibody yields are obtained by administering a series of injections (Cruickshank *et al.*, 1968). In our study the hyperimmune serum against ND virus was raised using series of injections following the schedule described researchers for raising of hyperimmune serum against NDV in rabbits (Reylond and Maraqa, 2000; Iqbal *et al.*, 2003). In our study chicken was used for raising of hyper immune serum. It is also recorded that vertebrate species ranging from farm animals to rabbits, small laboratory rodents and chickens can be used to raise hyper immune serum (Carpenter, 1975). Hussain *et al.* (2004) used series of injections of Infectious Bursal Disease virus for production of hyperimmune serum against IBD virus in rabbits and got maximum indirect haemagglutination titer (IHA) after 3rd and 4th inoculation. As the studies have reported that various factors play a major role in protective and fruitful immune response, e.g. antigenicity of vaccinal agent, age of immunological maturity in birds, physiological and environmental influences which include immuno-deficient problems, hormonal factors, stress poor nutrition, climatic factors, concurrent diseases and other chemical immunosuppressive agents (Hudson *et al.*, 1974). Considering all these factors the standard conditions of

management and housing were provided to chicks which were kept for raising of HIS in order to keep minimum stress on birds in our study.

In our study the LaSota strain in combination with Incomplete Freund's adjuvant was used to raise the sera in birds. The antibody formation is enhanced by use of certain adjuvant substances. Adjuvants are supposed to prolong the exposure of antigen to the immune system, protecting it from degradation and enhance the immune response by attracting and stimulating the immune stem cells (Jennings, 1995). Incomplete Freund's adjuvant is a water-in-oil emulsion of mineral oil surfactant and does not cause considerable pain in experimental animals (Jurd and Hansen, 1990). The combination of antigen and IFA also remained under the study of (Iqbal *et al.,* 2003). Live antigen containing incomplete Freund's adjuvant provided better immune response and may be a good choice for raising HIS (Hussain *et al.,* 2004). The antigen and adjuvant permits much smaller use of antigen and enhances the antibody titer compared with antigen without adjuvant (Kaeberle, 1986).

In our study antiserum was purified as three successive precipitations by ammonium sulphatesolution according to the method described by Villegas (1986). Scientists also used the same solution for the purpose of purification of antisera (Lyerla and Hierholzer, 1975; Swaminathan *et al.,* 1978; Bozhilov *et al.,* 1979).

In our study the experimental chicks in all groups were infected with 0.1mL of Velogenic strain of NDV (ELD_{50} = $10^{-5.33}$/0.1mL) through subcutaneous route and two hours post infection the birds were injected with HIS. Group D was injected with 6mL HIS through i.m route that provided protective level of antibodies for passive immunization in experimental group. It is supported by the experiments of previous study in which 0.5cc of HIS was administered through subcutaneous route in chicks after infection of chicks with ND virus at 24hr, 48hr and 70hr failed to provide protective level against ND virus infection (Moynihan *et al.,* 1954).

Newcastle disease having a devastating effect on economical poultry production (Alexander, 1980) has a vide host range and is responsible for high morbidity and mortality in susceptible birds of all age groups (Calnek, 1991). NDV differs in virulence and has been grouped into 5 pathotypes: viserotropicvelogenic, neurotropic velogenic, mesogenic, lentogenic and asymptomatic enteric (Beard and Hanson, 1984). Velogenic strain of ND virus may cause 100% mortality while mesogenic strain causes moderate disease and reduction in egg production (Alexander, 1997). ND is mostly caused by velogenic strains of NDV than mesogenic or lentogenic strains. The disease produced by velogenic strain may cause mortality ffrom 80 – 90% in adults (Eisa and Omer, 1984; Claudia *et al.,* 1996). The severity of disease varies greatly, spanning from per-acute disease with almost 100% mortality to subclinical disease with no leisions (Alexander, 1998) Similarly, in our study the birds were infected with velogenic strain of ND virus and the decreasing rate of mortality and morbidity was observed in groups passively immunized with increasing dose of HIS while high rate of mortality was observed in the groups not passively immunized with HIS after experimental infection of NDV. Researchers also reported that with Velogenic NDV the mortality can easily reach 100% and in experimental conditions the course of disease is rapid usually 2-4 days (Brown *et al.*, 1999; Kommers *et al.,* 2003; Kommers *et al.,* 2002; Wakamatsu *et al.,* 2006).

In our study the wide range of clinical signs and symptoms of ND were observed in groups of birds experimentally infected with NDV and passively immunized with different doses of HIS against NDV (Table III). Researchers have also reported that the velogenic strains of NDV cause respiratory and enteric signs in chicks along with conjunctivitis, difficult breathing and ruffled feathers (Kommers *et al.,* 2002; Oladele *et al.,* 2005), torticolis, blindness and wing paralysis (Brown *et al.,* 1999; Kommers *et al.,* 2002), diarrhea, nervous signs, shivering and paralysis of legs and wings in clinically affected pigeons due to ND (Shaheen *et al.* (2005). The major clinical signs of ND are depression, weakness, loss of appetite, dehydration, inability to stand, cyanosis of comb and wattle, greenish watery diarrhea, nasal and eye discharges, decreased egg production, loss of weight followed by death (Pazhanivel *et al.,* 2002).

There are many factors which effect severity of clinical signs of ND in birds mainly age, route of infection, immune status and concomitant environmental stress. Younger birds tend to have more severe and acute disease than older animals, intravenous inoculation is more likely to elicit neurologic signs and aerosolization of high viral doses tends to impact upper respiratory infection (Alexander, 1995). There is also species variation regarding expression of clinical signs in birds. The clinical signs are first recognizable starting at 2 days post inoculation (DPI) (Brown *et al.,* 1999; Kommers *et al.,* 2003; Kommers *et al.,* 2002; Wakamatsu *et al.,* 2006), birds had become off feed and dull after experimental infection of ND on 3rd DPI (Oladele*et al.,* 2005), and chicks had become severely depressed and inactive with hard ruffled feathers on 4th DPI, prostrated position and open mouth breathing on 5th DPI (Kommers *et al.,* 2002), nervous signs in sense of blindness and torticulus and incoordination on 7th and 10th DPI (Kommers *et al.,* 2002; Brown *et al.,* 1999). In one study birds also became anorexic, suffered from greenish diarrhea, and exhibited respiratory signs with nasal discharge and difficult breathing along with torticulus and in-coordination from 4th DPI in experimentally infected birds against NDV (Shahzad *et al.,* 2011). In another study the pigeons developed ND after day five post infection (pi) and birds became dull, anorexic and had droopy and paralyzed wings (Oladele *et al*., 2008). The clinical signs of ND observed in our study are in agreement with the previous

findings in turkeys (Abdul-Aziz and Arp, 1983; Adair *et al.,* 1989), guinea fowls (Haruna *et al.,* 1993; Mohammad *et al.,* 1996), quails (Oladele *et al.,* 2008a) and chickens (Alexander, 1997; Sa'idu *et al.,* 2006; Oladele *et al.,* 2008b). In present study mild production of disease in chicks of group C and D in this study was due to passive immunization provided by the HIS. Researchers have also described that the use of monoclonal Abs in passive immunization after challenge with virulent strain of NDV has protective effect against ND in chicks (Umino *et al.,* 1990).

In our study the leisions of ND were observed in groups of birds experimentally infected with NDV and passively immunized with different doses of HIS against NDV. Researchers have reported that lesions due to ND includehemorrhagic larynx, hemorrhagic trachea, congested lungs and cloudy air sacs containing exudates (Crespo *et al.,* 1999; Kommers *et al.,* 2002; Oladele *et al.,* 2002; Piacenti *et al.,* 2006), multi focal hemorrhages in the mucosa of proventriculus, caeca and small intestine of infected birds (Kommers *et al.,* 2002). In some studies the leisions of ND were not observed in birds up to 5 DPI (Brown *et al.,* 1999; Kommers *et al.,* 2002; Oladele *et al.,* 2002). As reported in our study the multi focal linear hemorrhages and ulcers in digestive tract including oral cavity, esophagus, proventriculus and intestine of games chicken infected with NDV were also observed by other scientists (Kommers *et al.,* 2002). Similar changes were observed in chicks (Alexander *et al.,* 1997) and necrotic spots on kidneys, pin point hemorrhages on heart and proventriculus were observed in clinically affected pigeons due to ND (Shaheen *et al.,* 2005). Similar findings were observed in chicks (Piacenti *et al.,* 2006) and multi focal linear hemorrhages in the larynx and trachea of game birds (Crespo *et al.,* 1999) infected with NDV. Barton *et al.* (1992) observed mild air sacculitis and focal pulmonary consolidation and caseousplaquesin trachea in pigeons infected with NDV. Nervous lesions including congestion and oedema in brain and torticolis were also observed in pigeon (Shaheen *et al.,* 2005) but any gross change in brain of game chicken was not observed (Crespo *et al.,* 1999) infected with NDV. Tracheal hemorrhages, spleenomegaly, alveoli filled with edematous fluid and infiltrated with mononuclear cells on 4^{th} DPI and lymphocyte degeneration in bursa and lymphoid hyperplasia in spleen on 8^{th} and 10^{th} DPI have also been recorded in experimentally infected birds against NDV (Shahzad *et al.,* 2011). In one study the muscles of breast, thighs and legs of pigeons that died due to ND were congested, carcasses were emaciated, dehydrated and had leisions of ND in various organs (Oladele *et al*., 2008). The gross leisions of ND are petechial hemorrhages and ulcers with raised borders on the mucosa of proventriculus, pneumonic lungs, and hemorrhages in trachea air sacs, brain and spleen (Pazhanivel *et al.,* 2002). The gross leisions of ND observed in our study are in agreement with the previous findings in turkeys (Abdul-Aziz and Arp, 1983; Adair *et al.,* 1989), guinea fowls (Haruna *et al.,* 1993; Mohammad *et al.,* 1996), quails (Oladele *et al.,* 2008a) and chickens (Alexander, 1997; Sa'idu *et al.,* 2006; Oladele *et al.,* 2008b).

In our study the wide range of histo-pathological changes in different organs were observed 5^{th} to 14^{th} DPI in different groups receiving different doses of HIS. Similarly, researchers observed congestion, micro-hemorrhages and sloughed epithelium in lamina propria of trachea from 6^{th} to 10^{th} DPI, lymphocyte infiltration in alveoli of lungs on 6^{th} DPI, congestion and hemorrhages in parabronchial region and inflammatory exudation in alveoli on 8^{th} and 10^{th} DPI (Shahzad *et al.,* 2011), epithelial hyperplasia of trachea on 4^{th} DPI, congestion of lungs, alveoli filled with edematous fluid and infiltration of mononuclear cells along with severe pulmonary congestion on 5^{th} DPI (Kommers *et al.,* 2002) and lymphoid hyperplasia on 5^{th} DPI lungs (Brown *et al.,* 1999) in experimentally infected birds with ND virus.

In our study high values of GMT were found in groups receiving higher doses of HIS as compared to groups receiving low doses of HIS and control groups. The higher level of GMT provided better protection in chicks against ND virus infection. In previous study the GMT of ND vaccinated broiler flocks based on age was determined and GMT values for age (week) group 0-3, 3-5 and 5-7 were 11.91, 10.01 and 15.85, respectively, and low level of antibodies production indicated the unsatisfactory level of protection in broilers against NDV infection (Numan, 2005).

In our study it was observed that HIS can be raised against NDV in chicks can be used to decrease the morbidity and mortality rate in experimentally infected birds.

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