

SEROPREVALENCE OF NEWCASTLE DISEASE VIRUS IN COMMERCIAL BROILER CHICKEN; MANSEHRA, PAKISTAN

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Abstract. This study was conducted to observe the serological status of Newcastle Disease Virus in broiler birds. A total of 248 samples from different age groups (0-3, 3-5, 5-7 week) of vaccinated (n=144) and non-vaccinated (n=104) broiler birds were collected from different areas of District Mansehra. The samples were analyzed at Veterinary Research and Disease Investigation Centre (VR&DIC), Abbottabad. Haemagglutination inhibition (HI) test revealed that 84% of samples were sero-positive in non vaccinated flock while 16% of samples were found negative for NDV antibodies. The effect of age on antibody titer in non vaccinated flocks was also noticeable which increased with age of the bird *viz*: 1-3 weeks 75%, 3-5 weeks 82% & 5-7 weeks 92.5% for NDV antibodies. On the other hand 97% of vaccinated broiler flocks were sero-positive while only 3% were negative which were from lesser age group (1-3 week). Such a high prevalence (84%) of NDV infection in non vaccinated broiler poultry flocks is a future risk for the outbreak of disease in the broiler and all other bird species including breeders and domestic/rural poultry. The level of protection of the vaccinated birds was also found unsatisfactory which indicate that present strategies are not fully effective in case of this infection.

Keywords: Seroprevalence, newcastle disease, broilers, haemagglutination.

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1. Introduction

In Pakistan the poultry sector is playing an important role in bridging the gap between the supply and demand for protein. Commercial poultry farming started in Pakistan in the early 1960's and showed rapid growth over the last few decades. The growth of this sector was the result of the promotional policies from the Government and the persistency of the poultry farming community. In Pakistan it is one of the most dynamic and well organized sector contributing 26.8% to total meat production. Its contribution in agriculture and livestock is 6.4 percent and 11.5 percent, respectively (Economic Survey of Pakistan 2011-12).

Over four million broiler breeder stock is annually reared in Hazara region, in addition to 2.5 million broilers, 0.5 million commercial layers and 3.00 million rural poultry (Ayaz *et al.*, 2010). District Mansehra is richly populated with commercial poultry farms due to its favorable climatic conditions.

Infectious diseases are among the main factors that affects poultry industry. In Pakistan, poultry industry faces a number of disease challenges which cause huge economical losses due to high rate of mortality, morbidity and decreased production egg (Alexander, 2000). Among these, Newcastle disease is a major threat for both

commercial and backyard poultry farming including all ages of chickens (Ghaniei *et al.*, 2012).

The Newcastle disease (ND) is a highly infectious and fatal viral disease, which not only affects domestic but also wild avian species (Alexander *et al.*, 2003). The disease is caused by Avian Avulavirus 1 (AAvV-1) (formerly as Avian Paramyxovirus) which is a member of the genus Avulavirus of family Paramyxoviridae and order mononegavirales. The genome of this family contains non-segmented, negative-sense single stranded RNA (-ive ssRNA) (Fauquet *et al.*, 2005, Mayo, 2012). NDV is divided into three different pathotypes on the bases of their virulence in poultry. These pathotype include lentogenic, velogenic and mesogenic forms (OIE, 2012, Alexander, 2003).

It is a complex disease affecting respiratory, digestive and nervous systems. Prominent signs include sneezing, coughing, greenish watery diarrhea, torticollis and dropping of wings. Other signs and symptoms include swelling of tissues around neck and eyes, dragging legs and loss of egg production (Wakamatsu *et al.*, 2006). The mortality rate may range between 30 to 100% in infected birds. No treatment is available but only prophylaxis and hygienic methods are used which may reduce the chance of outbreak & mortality.

Therefore, the current study was designed to know about its sero-prevalence of Newcastle Disease Virus in District Mansehra in order to devise a proper strategy to combat its harmful impacts on socio-economic condition of farmers and the country.

The aims of the current study were to determine the serological status of Newcastle Disease Virus in vaccinated and non-vaccinated flocks of broiler chicken with different age groups.

2. Materials and method

The current research work was done at Veterinary Research and Disease Investigation Centre (VR&DIC) Abbottabad, Pakistan. The blood samples were randomly collected from broiler flocks of District Mansehra

Collection of samples

A total of Thirty (30) commercial broiler flocks were selected. The farms were divided into three age groups & two further groups on the basis of vaccination. Blood samples ($n=248$) were collected in Gel added tubes. 144 blood samples were collected from 17 vaccinated farms, where as 104 blood samples were collected from 13 non vaccinated farms. The data was recorded *viz*; age of birds, vaccination and general signs and symptoms. Blood samples were shifted to Virology Lab in cold chain. Serum was separated by centrifugation for 10 minutes at 3000 rpm. The serum was collected in the Eppendorf tubes from centrifuged samples, labeled and stored at -20°C for further lab analyses.

Heamagglutination (ha) test

Procedure for ha assay

1. 0.025 ml of PBS (0.01 M) was dispensed in each well of V-bottomed micro titer plate.
2. 0.025 ml of virus suspension (NDV) was dispensed in the first well & twofold dilution was made across the plate.

3. and 12th well was left as a control. i.e., only PBS and RBCs were present in that well, again 0.025 ml of PBS was dispensed into each well.
4. 0.025 ml of 1% (v/v) chicken red blood cells was added into each well.
5. The microtitre plate was gently tapped to mix the solution in the wells. Allow the RBC for 40 minutes to settle at room temp i.e. 20°C.
6. HA was determined by tilting the plate for observing presence or absence of streaming. The titration was read at highest dilution (no streaming), this was considered as 1 HA unit and was calculated from initial dilutions.

Haemagglutination inhibition (hi) test

1. After labeling, 0.025 ml of PBS was taken in each well of V-bottomed plastic microtitre plate.
2. Through micropipette 0.025ml of serum was added in the first well.
3. Two fold dilutions of 0.025 ml of the serum were made across the plate.
4. 4 HA unit antigen in 0.025 ml was added in each well and plate was left for 30 minutes at room temperature i.e. 20°C
5. 0.025 ml of 1% (v/v) chicken red blood cells was added. After gentle mixing, the RBC's were allowed for 40 minutes to settle at room temperature.
6. Agglutination was observed by tilting the plates & only those wells in which RBC's stream at the same rate as that of positive control were considered to show inhibition. Antibody titer equal or greater to 4Log₂ was taken as positive.

3. Results

The results obtained from current study are elaborated in this chapter.

3.1 Haemagglutination pattern of serially diluted NDV

Agglutination appears as a mat of RBC covering the bottom of the well. Haemagglutination occurred up to 8th well in vaccinated birds whereas 9th to 11th well there was no agglutination. In non-vaccinated bird's agglutination occurred up to 6th well, whereas from 7-11 showed no agglutination. 12th well was used as a control. Haemagglutination/inhibition pattern was used to calculate HA/HI titer.

3.2 Sero-Prevalence of NDV in different Age Groups

3.2.1 1-3 week age group

Three non vaccinated poultry farms were visited and 24 blood samples were collected out of which 18 were Sero-positive (75%) while 6 were sero-negative (25%) as shown in Table 1.

Table 1. Serological status of non vaccinated broiler birds for NDV

Age of birds	No. of samples	No. of +ive samples	No. of -ive samples	% age of +ive samples	% age of -ive samples
1-3 week	24	18	6	75	25
3-5 week	40	33	7	82	18
5-7 week	40	37	3	92.5	7.95
Total	104	88	16	84	16

Four ND vaccinated farms were visited and 32 blood samples were collected. 88% of the samples were sero-positive while 12% were sero-negative as shown in Table 2.

3.2.2 3-5 week age group

Forty samples were collected for the age group ranging from 3-5 week from Five non vaccinated broiler farms. Out of which 82% samples were sero-positive (n=33) for NDV while 18% (n=7) was found sero-negative, as shown in Table 1.

About 56 blood samples from seven vaccinated farms were also collected for this age group (3-5 week). All the samples were sero-positive for NDV with 100% of sero-prevalence as shown in Table 2.

Table 2. Serological status of vaccinated broilers birds for NDV

Age of birds	No. of samples	No. of +ive samples	No. of -ive samples	% age of +ive samples	% age of -ive samples
1-3 week	32	29	3	90.62	9.38
3-5 week	56	56	None	100	None
5-7 week	56	56	None	100	None
Total	144	141	3	97	3

3.2.3 5-7 week age group

Forty samples from non vaccinated birds between 5 to 7 week of age were collected from four farms. Only three samples (7.95%) were sero-negative and remaining all samples were sero-positive for (92.5%) NDV as shown in Table 1.

Similarly 56 blood samples were collected from vaccinated chicken. All of these samples were sero-positive (100) as shown in Table 2.

3.3. Haemagglutination Titers:

The titers of blood samples are recorded in table 3 and 4. The highest titration was $10\log_2$, while $2\log_2$ was the lowest value. Titration $2\log_2$ or below $2\log_2$ were recorded as negative because they produced no antibody against the virus. Samples were considered positive for NDV antibodies that had NDV antibody titer $4\log_2$ and above.

Table 3. Haemagglutination inhibition titers of different age group of Non-vaccinated broiler poultry against Newcastle disease virus

Age (weeks)	No. of samples	Antibody titers by using HI test									
		\log_2	$2\log_2$	$3\log_2$	$4\log_2$	$5\log_2$	$6\log_2$	$7\log_2$	$8\log_2$	$9\log_2$	$10\log_2$
0-3	24	4	2	5	4	6	3				
3-5	40	2	6	6	7	8	5	1	3	2	
5-7	40		3	4	3	7	5	4	4	6	4

In non vaccinated flock, out of the 104 samples tested, 16 (15.38%) birds were negative for NDV antibody, because they had the antibody titer below the minimum titer of $3\log_2$ (i.e. 4 birds had NDV antibody titer of \log_2 and 2 birds had titer of $2\log_2$).

The remaining chickens were positive for NDV antibody having NDV antibody titer $4\log_2$ and above (Table 3.).

Table 4. Haemagglutination inhibition titers of different age group of vaccinated broiler poultry against Newcastle disease virus

Age (weeks)	No. of samples	Antibody Titers by using HI test									
		Log_2	$2\log_2$	$3\log_2$	$4\log_2$	$5\log_2$	$6\log_2$	$7\log_2$	$8\log_2$	$9\log_2$	$10\log_2$
0-3	32	1	2	5	9	6	5	4			
3-5	56			7	6	4	5	9	13	5	7
5-7	56			1	4	5	3	14	16	6	8

In vaccinated flock, 144 serum samples were tested out of which 141 (97%) birds were sero-positive for NDV antibodies. Results in table 3.4 indicate a higher percentage of the chickens (54%) had protective NDV antibodies between $6\log_2$ and $8\log_2$.

4. Discussion

Newcastle disease virus has been reported including to cause huge losses of poultry industry throughout the world causing deadly disease in chickens, turkeys and many other birds. The serological tests are very appropriate for surveillance and early detection of NDV infection. In this respect four major tests are used i.e. Enzyme Linked Immunosorbent Assay (ELISA), Haeme-agglutination Inhibition (HAI), agar gel immune diffusion and Polymerase chain Reaction (PCR). For early detection of infection, HAI test is the most suitable test. Hence in the current study HAI was used to find the serological status of Newcastle Disease Virus.

This study was conducted to observe the serological status of Newcastle Disease Virus in broiler birds. A total of 248 samples from different age groups (0-3, 3-5, 5-7 week) of vaccinated (n=144) and non-vaccinated (n=104) broiler birds were collected from different areas of District Mansehra. Haemagglutination inhibition (HI) test revealed that 84% of samples were sero-positive in non vaccinated flock while 16% of samples were found negative for NDV antibodies. Such a high prevalence (84%) of NDV infection in non vaccinated broiler poultry flocks is a future risk for the outbreak of disease in the broiler and all other bird species including breeders and domestic/rural poultry. This heavy titer of poor vaccine quality, improper storage and use of expired vaccine, or improper dispensing might result in out breaks in vaccinated (Vui *et al.*, 2009, Sil *et al.*, 2002). Immuno-suppression of birds occurs due to steroids administration, heat stress and water deprivation which further adds to disease outbreak (Rehman *et al.*, 2017). Also poor nutrition like hypo-proteinemia may harm the immune response.

The effect of age on antibody titer in non vaccinated flocks was also noticeable which increased with age of the bird *viz*: 1-3 weeks 75%, 3-5 weeks 82% & 5-7 weeks 92.5% for NDV antibodies. The results of this study are in line with the study which

showed that maximum protection was found in birds between the age of 3-7 weeks (Rezaeianzadeh *et al.*, 2011).

In a survey conducted in field in which it was concluded that 61.9% bird were sero-positive (Hadipour, 2009). Likewise, in another study it was found that 37.56% chicken were sero-positive in field (Mozaffor Hossain *et al.*, 2010). In Bangladesh it was reported that a total 78.04% broiler birds were sero-positive for NDV antibodies (Siddique *et al.*, 2005).

On the other hand 97% of vaccinated broiler flocks were sero-positive while only 3% were negative which were from lesser age group (1-3 week).

During the study it was also observed that in non vaccinated birds also shown antibodies for NDV infection. The detectable level of antibodies against NDV shows that birds were naturally exposed to virus. Moreover, in vaccinated flocks of Pakistan a number of outbreaks of NDV have been reported. Many field studies in past indicated that outbreaks in vaccinated birds, occur due to new viral strains & improper vaccination practices (Dortmans *et al.*, 2012).

5. Conclusions

In conclusion, the presence of antibodies in non-vaccinated flocks was alarming because such challenge of virus usually results in outbreaks resulting heavy mortality. Moreover, the level of protection of the vaccinated birds was also found unsatisfactory which may be due to improper dosing & improper storage of vaccine.

It is therefore recommended to prevent the outbreak of NDV infection, strict bio-security measures should be adopted. Bio-security measures and continual boosting of birds immunity with NDV vaccine should be practiced.

In poultry farm the movement of wild birds must be controlled because these birds not only shed virus but also spread it from farm to farm. The morbid material of poultry should be burned or properly dumped.

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