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Immune Status Against Newcastle Disease Virus in Backyard Poultry of Punjab Province, Pakistan

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Abstract | Newcastle Disease (ND) is one of the most important health problem of commercial and backyard poultry. It is producing significant economic losses due to fatalities globally. The objective of the study was to carried out serum base investigation regarding the Newcastle disease Virus (NDV) antibody occurrence in different seasons of a year. The data were documented has current immune status of unvaccinated backyard poultry. A total of 1468 sera samples were collected from 32 districts of Punjab, Pakistan. Using Haemagglutination inhibition (HI) test, the serum Ab titer was measured and maximum seroprevalence (92%) was discovered in the Sargodha district, whereas Mianwali showed lowest seroprevalence (15.25%). Overall seropositivity (58.78%) was observed between $4\log_2$ and $9\log_2$ antibody titer and average geometric mean titer (GMT) was 16.59. Furthermore, the presence of serum base antibodies of NDV was found higher in summer season (64.50% with 21.38 GMT) but decreased in winter season (50% with 15.13 GMT). In the absence of the vaccine, the results provide a clear evidence of exposure to NDV to backyard poultry. Hence, systemic sero-surveillance is needed to counter the ever-increasing risk of NDV in backyard poultry.

Keywords | Newcastle disease virus (NDV), Backyard poultry, Antibody titer Haemagglutination inhibition (HI) test, Punjab, Pakistan

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INTRODUCTION

ND causes a large number of outbreaks and economic losses in poultry industry throughout the world. (Alexander, 2001). Due to its tremendous economic losses and huge epidemics, World Organization for Animal Health (OIE) has listed it among the diseases that require immediate reaction upon recognition (OIE, 2012). This viral disease is extremely contagious and attacking several species of domestic and wild birds (Alexander et al., 2012). ND is caused by negative-sense single stranded RNA virus "avian paramyxovirus serotype-1 (APMV-1)" of genus *Avulavirus* under order *Mononegavirales* within the family *Paramyxoviridae* (King et al., 2011). NDV is divided into five pathotypes based on the severity of disease in chickens and these are namely, viscerotropic velogenic, neurotropic velogenic, lentogenic, mesogenic, and asymptomatic enteric form (Ahmed and Odisho, 2018).

ND is characterized by producing reproductive, digestive, nervous, and respiratory problems (Ashraf and Shah, 2014). Clinical signs depend upon various factors such as age of host, host species and strain of virus. Immune system, secondary infection and environmental stress also play vital role in disease progression (Al-Habeeb et al., 2013). Chickens show symptoms of anorexia, abnormal thirst, weakness and low egg production. In case of respiratory signs, there is nasal discharge along with cough, while

digestive form includes green water diarrhea. Twisting of head and neck and paralysis of wings or legs is observed in nervous form (Bhaiyat et al., 1994). The NDV infect several species of domestic and wild birds regardless of variation in sex and age (Alexander et al., 2012; Iram et al., 2014).

Being an agro-country, livestock and poultry production are major profitable outcomes in the form of GDP in Pakistan. Pakistan has become the 11th largest poultry producer in the world with the production of 1,163 million broilers annually. Employment of more than 1.5 million people is linked to this industry. Around 89.84 million domestic poultry exist in Pakistan which occupies a place of pride among livestock enterprises due to its rapid monetary revenue (Economic Survey of Pakistan, 2019-20). In rural areas of many developing countries, poultry rearing is widely practiced (Alders et al., 1994) and considered as an important asset for a substantial source of protein in the form of eggs and meat. The seroprevalence study has revealed the existence of ND in backyard poultry in numerous countries (Couacy-Hymann et al., 2012; Molia et al., 2017; Alsahami et al., 2018). In Pakistan, results showed that ND is prevalent in domestic birds (Aziz-ul-Rahman et al., 2017) as major poultry hubs are located and growing in Punjab, Pakistan. Therefore, regulatory framework and monitoring services are needed to prevent health issues of poultry in concerned areas. The present study was conducted to evaluate the incidence of antibodies against NDV in backyard poultry which will help us to investigate epidemiology of virus in backyard and commercial poultry.

MATERIALS AND METHODS

Study area

Punjab province (31°N 72°E) is located along the northwestern edge of the geologic Indian plate in South Asia. Punjab province is located 139 m above sea level, and it is the most populous province (approximately 56% population of the Pakistan lives in this region). The 32 districts (mentioned in Table 1) was selected to investigate the current incidence of antibodies against NDV in backyard poultry. Through a serological survey of a one year, a total of 1468 blood samples of unvaccinated backyard poultry were obtained randomly from 32 districts of Punjab, Pakistan. About 5ml of blood was collected aseptically from wing vein and permitted to clot.

Haemagglutination inhibition (HI) test

To determine the antibody titer against ND virus, this test was performed using a reference strain of ND as control antigen obtained from Veterinary Research Institute (VRI), Ghazi Road Lahore. The blood from the chickens were taken and centrifuged at 1500 rpm for 10 minutes. HI test was performed according to the protocol described by (Alexander and Chettle, 1977) by making 2-fold serial dilution of serum samples using 4HA unit of NDV. The antibody titer of serum samples was measured by observing the button formation due to settling of RBC's. The HI titer was determined as per standard operating procedures directed by manual published by OIE. Haemagglutination inhibition was considered as an end point at which sera were at maximum dilution. Samples were categorized as seronegative if sera had titer of <1:16 and sera with >1:8 titers were considered to be positive (Musa et al., 2009).

Statistical analysis

For descriptive statistics, collected data were entered in computer program MS EXCEL (Microsoft Co.). Geometric mean titer (GMT) of HI was calculated as designated by (Brugh et al., 1978) and to assess the immunity status, mean based univariate analysis was done through ANOVA in IBM SPSS ®.

RESULTS AND DISCUSSION

Overall, 1468 blood samples from different districts were tested by using the HI test. The antibody titer was observed up to 1:512. Most of the birds showed antibody titer up to $9\log_2$. The average geometric titer for all districts was 16.59. The overall seroprevalence of ND in all districts of Punjab was 59%. These results are shown in Table 1.

The GMT titer and seroprevalence from age 20 weeks to >65 weeks was in range from 55.42% to 62.10%. The antibody titer was abundant in older birds as compared to young ones. These results showed that as age increased the GMT titer were also increased. Among four different groups, chickens with age 65 weeks showed high seroprevalence (62.10%) followed 51-65 weeks (58.69%), 36-50 weeks (58.29%) old birds and least seropositive were birds of age 20-35 as shown in Table 2.

The seroprevalence and GMT titer were also observed in different seasons in Punjab. The anti-NDV antibodies showed high existence in sera collected during summer season (64.50%) as compared to other seasons, 56.39% in autumn, 50% in winter and 63.79% in rainy seasons with 21.38, 16.22, 15.13 and 17.38 GMT, respectively as shown in Table 2.

Pakistan is endemic with NDV poultry because the virus prevailing not only in poultry (Munir et al., 2012) but also in other birds (Alexander et al., 2012). HI antibody titer against ND of $3\log_2$ and above is commonly considered as positive for specific immunity (Allan and Gough,1974). Here is a conflict, because some authors used ≥ $2\log_2$ (Biswas et al., 2009), $3\log_2$ (Tadesse et al., 2005) and $4\log_2$

Table 1: Overall results on geometric mean titer to NDV in backyard poultry reared in 32 District in Punjab.

Sr.	Name of districts	Sample size	Log_2 HI titre to NDV									GMT	% Occurrence
No.			1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512		
1	Multan	50	8	11	12	4	-	5	7	3	-	13	38%
2	Khanewal	50	11	17	2	6	5	-	9	-	-	9.57	40%
3	Jhang	50	6	10	13	1	12	8	-	-	-	11.64	42%
4	Muzaffar Garh	50	6	-	9	11	16	3	-	5	-	19.68	70%
5	Hafiz Abad	50	-	10	1	11	19	2	7	-	-	21.97	78%
6	Rajan Pur	50	3	1	2	17	13	8	5	-	1	26.3	88%
7	Narowal	50	2	11	3	19	1	-	9	5	-	20.23	68%
8	Bahawal Pur	27	3	5	1	10	4	4	-	-	-	36.39	66.67%
9	Bahawal Nagar	57	-	10	7	-	16	11	6	6	1	32.36	70.18%
10	Sheikhupra	50	9	-	2	9	6	21	3	-	-	23.6	78%
11	Okara	42	-	17	-	11	-	5	8	1	-	17.1	59.52%
12	Layyah	46	6	4	3	3	16	1	10	3	-	25.53	71.74%
13	Sargodha	50	4	-	-	14	5	9	11	7	-	43.34	92%
14	Gujranwala	61	3	19	12	8	10	1	2	2	4	9.59	44.26%
15	Pakpattan	50	-	21	9	12	-	7	-	1	-	10.12	40%
16	Khushab	13	1	-	3	-	8	1	-	-	-	19.82	69.23%
17	Gujrat	60	-	12	12	10	9	15	2	-	-	17.74	60%
18	Chiniot	51	17	13	9	1	2	2	5	1	1	7.46	24%
19	Bhakkar	50	10	3	-	6	14	7	7	3	-	22.59	74%
20	Lodhran	31	1	9	11	4	-	-	5	1	-	11.97	32.26%
21	Sahiwal	34	-	11	7	6	5	-	-	3	2	15.35	47.05%
22	Sialkot	50	-	21	1	3	5	1	9	10	-	24.55	56%
23	Vehari	50	-	24	13	9	1	3	-	-	-	7.57	26%
24	Faisal Abad	50	5	16	8	8	5	6	-	2	-	10.54	42%
25	Attock	50	6	-	-	19	11	5	6	3	-	25.29	88%
26	Mianwali	16	2	9	1	1	3	-	-	-	-	6.16	15.25%
27	Jehlum	50	13	7	11	8	2	4	5	-	-	9.31	60%
28	Toba Tek Singh	39	8	1	2	1	8	8	6	3	2	30.34	71.79%
29	Kasur	50	3	2	7	4	5	19	6	4	-	36.22	76%
30	Rahim Yar Khan	50	-	6	29	9	5	1	-	-	-	9.97	30%
31	Nankana	35	2	-	4	4	7	13	3	2	-	35.32	71.42%
32	Dera Ghazi Khan	56	-	6	6	12	12	9	3	7	1	31.19	57.14%
Total		1468	129	276	200	241	225	179	134	72	12	16.59	58.78%

*Protective threshold; GMT: Geometric mean titer; NDV: Newcastle disease virus; HI: Hemagglutinin inhibition; C.I: Confidence Interval; NS: Non-significance.

Table 2: Age wise variation in observation of seropositivity against ND virus.

Age group (Weeks)	Sample size	Anti-NDV antibodies titer by HI test									GMT	Sero-prev alence	Mean ± SE	95% C. I
		2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9				
20-35	332	26	80	42	88	51	11	24	9	1	16.98	55.42%	30.67±13.52	-4.08; 65.41
36-50	422	33	77	66	71	65	58	31	19	2	17.38	58.29%	41.0±11.36	11.80; 70.20
51-65	305	29	59	38	36	43	47	25	23	5	19.49	58.69%	29.83±6.31	15.62; 59.04
>65	409	41	60	54	46	66	63	54	21	4	20.89	62.10%	42.33±10.10	16.37; 68.30

Table 3: Seasonal variation in observation of seropositivity against ND virus.

Season	Sample size	Anti-NDV antibodies titer by HI test									GMT	Sero-prevalence	Mean ± SE	95% C. I
		2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9				
Autumn	383	34	88	45	63	54	43	34	19	3	16.22	56.39%	36.0±9.09	12.61; 59.39
Winter	348	23	70	81	58	34	35	30	16	1	15.13	50.00%	29.0±7.87	9.78; 51.22
Summer	400	40	59	43	59	58	64	45	28	4	21.38	64.50%	41.67±9.10	18.27; 65.06
Rainy	337	32	59	31	61	79	37	25	9	4	17.38	63.79%	29.49±12.04	4.88; 66.78

*Protective threshold; GMT: Geometric mean titer; NDV: Newcastle disease virus; HI: Hemagglutinin inhibition; C.I: Confidence Interval.

(Gutierrez-Ruiz et al., 2000) as cutoff titer for antibody production against the virus. Chickens which are reared in villages can withstand the infection without showing any clinical symptoms, thus acting as a potential source of infection for commercial chicken (Ananth et al., 2008).

Based on the serological evidence of the present study, Sargodha showed 92% higher seropositivity against NDV as compared to Miawali (15.25%) with overall seroprevalence as 58.78% with high GMT (16.59) as mentioned in Table 1. As similar findings have been revealed in previous reports on rural or village poultry with high seroprevalence 62-72% in Nigeria (Ezeokoli et al., 1984). Our finding is in agreement of previous investigation in Pakistan, as 98.07% and 100% occurrence of specific immunity against NDV for broilers and layer, were evaluated respectively (Numan et al., 2005). The current study results are also consistent with previous findings regarding serological survey in backyard poultry from Zambia (36.9%); (Alders et al., 1994), Zimbabwe (27%); (Kelly et al., 1994), Tanzania (46.1%); (Yongolo et al., 2002) South Africa (5%); (Thekisoe et al., 2003), Central Ethiopia (32.2%); (Tadesse et al., 2005), Ecuador (97%); (Hernandez-Divers et al., 2006) and Bangladesh (88%); (Biswas et al., 2009).

Age distribution in testing sera from the chicken was another risk factor in our study, because the prevalence of antibody titer to NDV increased with increasing age (East et al., 2006). As similar finding, a previous study was observed high seroprevalence of NDV in rural chickens from southern part of Bangladesh (Biswas et al., 2006). Similar to our findings, chickens with age >65 weeks showed high GMT (44.86) followed by 53-64 weeks (41.80), 41-52 weeks (39.05), 29-40 (37.13) weeks and least prevalent was in 17-28 weeks (33.11) of age groups (Hossain et al., 2010). Chicks revealed GMT 20.2 and adults had GMT 26.3 (Kemboi et al., 2013). It supported the fact that the prevalence of antibody titer to NDV increased with increasing age.

Kemboi et al., 2013 revealed high geometric mean antibody titer of chicks in wet season as compared to dry season. GMT results were 70.7 and 20.2 in wet season and dry season respectively. The matter of the seasonal influence is still debatable and may differ according to the geographical, dietary and socioeconomic situations. The ND peaks mostly occur at the start of the rainy season in Vietnam (Nguyen, 1991). Current findings also revealed the high seroprevalence in rainy season. Prevalence of NDV antibody titer of serum samples in layer and broiler birds was evaluated. Broiler chicken had 90%, 79.39%, 75.81% and 64.35% HI titer against NDV in summer, autumn, winter and rainy season respectively. Similarly, Layer chickens had 98.35%, 97.98%, 97.46% and 92.86% HI antibody titer in summer, winter, autumn and rainy season respectively that provides us clue regarding increased antibody titer against NDV in summer season (Hossain et al., 2010). ND outbreaks are linked to the variation of seasons (Martin and Spradbrow, 1991) because of high wind movement transfers infection from one poultry house or flock to the other (Manchang et al., 2004). Investigation and control measure related to NDV transmission should be future planning in those areas where it is endemic.

NDV is still causing major economic losses to our meat and eggs in various parts of Punjab which needs due attention by provincial and national health authority and livestock officers to come up with control and prevention policy. As, serological analysis is unable to demonstrate the type and nature of NDV strains in field conditions. Hence, there is a need for further work to understand the circulating strains or pathotypes and its epidemiology. Second, proper vaccination program against NDV may improve the backyard poultry production and will be helpful in successful control of diseases. So, for implementation of a successful NDV vaccination program, several parameters should be evaluated. Now, further study concerns to virological investigation is need of time to understand the interactions of virulent or avirulent virus and their impact on the backyard poultry production system.

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AUTHOR'S CONTRIBUTION

All the authors have contributed in terms of giving their technical expertise to give a tenable shape to this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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