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 list 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 9log. 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 3log and above is commonly considered as positive for specific immunity (Allan and Gough,1974). Here is a conflict, because some authors used ≥ 2log₂ (Biswa et al., 2009), 3log₂ (Tadesse et al., 2005) and 4log₂, respectively.
| Sr. No. | Name of districts | Sample size | Log₂ HI titre to NDV | GMT | % Occurrence |
|---------|------------------|-------------|----------------------|-----|--------------|
| 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          | -                    | 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 |
|-------------------|-------------|--------------------------------------|-----|------------------|-----------|----------|
| 20-35             | 332         | 12                                   | 88  | 51               | 29        | 11       | 24.9  | 1       | 1.98 | 55.42% | 30.67±13.52 | -4.08; 65.41 |
| 36-50             | 422         | 12                                   | 88  | 51               | 29        | 11       | 24.9  | 1       | 1.98 | 55.42% | 30.67±13.52 | -4.08; 65.41 |
| 51-65             | 305         | 12                                   | 88  | 51               | 29        | 11       | 24.9  | 1       | 1.98 | 55.42% | 30.67±13.52 | -4.08; 65.41 |
| >65               | 409         | 12                                   | 88  | 51               | 29        | 11       | 24.9  | 1       | 1.98 | 55.42% | 30.67±13.52 | -4.08; 65.41 |
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 (Ezekokoli 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.

**ACKNOWLEDGMENT**

We would like to thank the Director of Veterinary Research Institute, Ghazi Road Lahore, Pakistan for providing laboratory facilities and helping in sampling.
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.

REFERENCES

- Ahmed A, Odisho SM (2018). Isolation identification and pathotyping of Newcastle disease viruses form naturally infected chickens in Iraqi Kurdistan region. Iraqi J. Agric. Sci., 49: 132-141.
- Al-Habeeb MA, Mohamed M, Sharawi S (2013). Detection and characterization of Newcastle disease virus in clinical samples using real time RT-PCR and melting curve analysis based on matrix and fusion genes amplification. World, 6(5): 239-243. https://doi.org/10.5455/verworld.2013.239-243
- Alders R, Inoue S, Katongo J (1994). Prevalence and evaluation of Hitchner B1 and V4 vaccines for the control of Newcastle disease in village chickens in Zambia. Pre. Vet. Med., 21(2): 125-132. https://doi.org/10.1016/0167-5877(94)90001-9
- Alexander DJ (2001). Newcastle disease. Br. Poult. Sci., 42(1): 5-22. https://doi.org/10.1016/S0007-1468(01)70014-4
- Alexander D, Chettle N (1977). Procedures for the haemagglutination and the haemagglutination inhibition tests for avian infectious bronchitis virus. Avian Pathol. 6(1): 9-17.
- Alexander DJ, Aldous EW, Fuller CM (2012). The long view: A selective review of 40 years of Newcastle disease research. Avian Pathol., 41(4): 329-335. https://doi.org/10.1080/03079459.2012.697991
- Almassi H, Ideris A, Omar A, Ramanoon SZ, Sadiq MB (2008). Isolation of Newcastle disease viruses of high virulence in unvaccinated healthy village chickens in south India. Int. J. Poult. Sci., 7: 368-373. https://doi.org/10.3923/ijps.2008.368.373
- Ashraf A, Shah M (2014). Newcastle disease: Present status and future challenges for developing countries. Afr. J. Microbiol. Res., 8(5): 411-416. https://doi.org/10.5897/AJMR2013.6540
- Aziz-ul-Rahman HM, Riaz T, Hussain B, Yousaf F, Saqalein M, Rasool M (2017). Seroprevalence of Newcastle disease virus (NDV) in commercial and domesticated birds: Pakistan during current surge of NDV. J. Infect. Mol. Biol., 4(4): 54-59. https://doi.org/10.14737/journal.jimb.2016.4.4.54.59
- Bhuiyant M, Ochiai K, Itakura C, Islam M, Kida H (1994). Brain lesions in young broiler chickens naturally infected with a mesogenic strain of Newcastle disease virus. Avian Pathol., 23(4): 693-708. https://doi.org/10.1080/03079459408419038
- Biswas P, Barua H, Uddin G, Biswas D, Ahad A, Debnath N (2009). Serosurvey of five viruses in chickens on smallholdings in Bangladesh. Prev. Vet. Med., 88(1): 67-71. https://doi.org/10.1016/j.prevetmed.2008.06.018
- Biswas P, Uddin G, Barua H, Roy K, Biswas D, Ahad A, Debnath N (2006). Immune status of semi-savaging Sonali chickens in Bangladesh against Newcastle disease. Livest. Res. Rural, pp. 18.
- Brugh Jr M (1978). A simple method for recording and analyzing serological data. Avian Dis., pp. 362-365. https://doi.org/10.2307/1589552
- Couacy-Hymann E, Kouakou A, Kouvame C, Kouassi A, Koffi Y, Godji P, Nana P, Tarnagda Z, Akoua-Koffi C (2012). Surveillance for avian influenza and Newcastle disease in backyard poultry flocks in Cote d’Ivoire, 2007–2009. Rev. Sci. Tech., 31: 821-828. https://doi.org/10.20506/rest.31.3.2158
- East I, Kite V, Daniels P, Garner G (2006). A cross-sectional survey of Australian chicken farms to identify risk factors associated with seropositivity to Newcastle disease virus. Prev. Vet. Med., 77(3-4): 199-214. https://doi.org/10.1016/j.prevetmed.2006.07.004
- Economic Survey of Pakistan (2019–20). Retrieved from http://www.finance.gov.pk/survey_1920.html
- Ezeokoli C, Unoh J, Adesiyun A, Abdu P (1984). Prevalence of Newcastle disease virus antibodies in local and exotic chicken under different management systems in Nigeria. Bulletin of animal health and production in Africa Bulletin des sante et production animales en Afrique.
- Gutierrez-Ruiz E, Ramirez-Cruz G, Gamboa EC, Alexander D, Gough R (2000). A serological survey for avian infectious bronchitis virus and Newcastle disease virus antibodies in backyard (free-range) village chickens in Mexico. Trop Anim. Health Prod., 32(6): 381-390. https://doi.org/10.1023/A:1005281619260
- Hernandez-Divers SM, Villegas P, Prieto F, Unda JC, Stedman N, Ritchie B, Carroll R, Hernandez-Divers SJ (2006). A survey of selected avian pathogens of backyard poultry in northwestern Ecuador. J. Avian Med. Surg., 20(3): 147-158. https://doi.org/10.1647/2005-015R.1
- Hussain KM, Ali MY, Yamato I (2010). Antibody levels against Newcastle disease virus in chickens in Rajshahi and surrounding districts of Bangladesh. Int. J. Biol., 2(2): 102. https://doi.org/10.5539/ijb.v2n2p102
- Iram N, Shah MS, Ismat F, Habib M, Iqbal M, Hasnain SS, Rahman M (2014). Heterologous expression, characterization and evaluation of the matrix protein from Newcastle disease virus as a target for antiviral therapies. Appl. Microbiol., 98(4): 1691–1701. https://doi.org/10.1128/AEM.00525-13
- Kelly PJ, Chitauro D, Rohde C, Rukwava J, Majok A, Davelaar F, Mason PR (1994). Diseases and management of backyard chicken flocks in Chitungwiza, Zimbabwe. Avian Dis., pp. 626–629. https://doi.org/10.2307/1592089
- Kemboi D, Chege H, Bebora L, Maingi N, Nyaga P, Mbuthia P, Njagi L, Githinji J (2013). Seasonal Newcastle disease antibody titer dynamics in village chickens of Mbeere District, Eastern Province, Kenya. Livest. Res. Rural, 25(10): 181.
- King AM, Leftkowitz E, Adams MJ, Carstens EB (2011). Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Elsevier.

January 2021 | Volume 9 | Issue 1 | Page 154
• Manchang T, Abdu P, Saidu L (2004). Epidemiology and clinicopathologic manifestations of newcastle disease in Nigerian local chickens: Epidémiologie et manifestations clinicopathologiques de la maladie de Newcastle chez des poulets de race locale nigérians: Epidemiologica y manifestaciones clinico-patologicas de la enfermedad de Newcastle en los pollos de raza local nigerianos. Revue d’élevage et de médecine vétérinaire des pays tropicaux. 57(1–2). https://doi.org/10.19182/remvt.9902

• Martin P, Spadlbrow P (1991). The epidemiology of Newcastle disease in village chickens, Newcastle disease in village chicken. Canberra, Australia. Austra. Cent. Int. Agric. Res., pp. 40–45.

• Molla S, Grosbois V, Kamissoko B, Sidibe MS, Sissoko KD, Traore I, Diakite A, Pfeiffer DU (2017). Longitudinal Study of Avian Influenza and Newcastle Disease in Village Poultry, Mali, 2009–2011. Avian Dis., 61(2): 165-177. https://doi.org/10.1637/11502-092616-Reg.1

• Munir M, Cortey M, Abbas M, Afzal F, Shabbir MZ, Khan MT, Ahmed S, Ahmad S, Baule C, Stahl K (2012). Biological characterization and phylogenetic analysis of a novel genetic group of Newcastle disease virus isolated from outbreaks in commercial poultry and from backyard poultry flocks in Pakistan. Infect. Genet. Evol., 12(5): 1010-1019. https://doi.org/10.1016/j.meegid.2012.02.015

• Musa U, Abdu P, Dafwang I, Umoh J, Saidu L, Mera U, Edache J (2009). Seroprevalence, seasonal occurrence and clinical manifestation of Newcastle disease in rural household chickens in Plateau State, Nigeria. https://doi.org/10.3923/ijps.2009.200.204

• Nguyen TD (1991). editor. Newcastle disease in village chickens control with thermostable oral vaccines. Proceeding (1991).

• Numan M, Zahoor M, Khan H, Siddique M (2005). Serologic status of Newcastle disease in broilers and layers in Faisalabad and surrounding districts. Pak. Vet. J. S., 25(2): 55.

• OIE U (2012). Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). See http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals.

• Tadesse S, Ashenafi H, Aschalew Z (2005). Seroprevalence study of Newcastle disease in local chickens in central Ethiopia. Int. J. Appl. Res. Vet. Med., 3(1): 25-29.

• Thekiso M, Mbati P, Bisschop S (2003). Diseases of free-ranging chickens in the Qwa-Qwa District of the northeastern Free State province of South Africa. J. S. Afr. Vet. Assoc., 74(1): 14-16. https://doi.org/10.4102/jsava.v74i1.490

• Yongolo M, Machangu AM, Minga U (2002). Newcastle disease and infectious bursal disease among free-range village chickens in Tanzania. Characteristics and parameters of family poultry production in Africa. IAEA, Vienna.