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# Prevalence, risk factors, haemato-biochemical profiles, and histopathology of Newcastle disease virus (NDV) in domestic pigeons, Pakistan

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## Abstract

*Newcastle disease (ND), caused by paramyxovirus serotype-1, is a highly transmissible and economically significant pigeon infection. The purpose of this study was to investigate the epidemiology of Newcastle disease and determine the effects of the Newcastle disease virus on the Haemato-biochemical profile and histopathology changes in domestic pigeons. A total of 50 blood, serum and tissue samples were collected from infected pigeons at sampling sites in Rawalpindi (42 lofts) and Gujar khan (100) lofts in Punjab, Pakistan. Data regarding associated risk factors were collected through predesigned form at the time of sample collection.*

*The virus was confirmed by haemagglutination inhibition test. Overall prevalence of ND disease was 18.45%, however it was highly significant in Gujar Khan 19.88%, as compared to Rawalpindi 14.68%. The infection was significantly higher (P = 0.000) in domestic pigeons as compared to racing, fancy and wild pigeon. The vaccinated pigeons had significant reduced infection rate in contrast to non-vaccinated. The clinical signs and pattern of diseases occurrence were also observed and showed significant variation among infected pigeons. Analysis of data of the associated risk factors revealed that age, gender, hygienic conditions, and locality of loft were non-significant with the incidence of disease. Various haematological parameters were also compared; the findings indicated that significant differences in lymphocyte, neutrophil, and erythrocyte sedimentation rate in clinically infected pigeons from the control group. When biochemical parameters were compared, the majority of the parameters under investigation were found to be non-significant. The histopathological analysis of naturally affected pigeons revealed non-suppurative inflammatory changes in brain, evident hydropic degeneration and fatty changes in hepatocytes, haemorrhages and congestion in intestine. The study findings helps to design a sustainable epidemiological approach to combat the NDV in pigeons.*

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## Introduction

Pigeon Paramyxovirus type 1 (PPMV-1) is an antigenic and host variant of Avian Paramyxovirus type 1 (APMV-1) or classical Newcastle Disease virus (NDV), which causes pathology and infection similar to Newcastle Disease (ND) in pigeons (Ujvári et al., 2003). *Columba livia* also known as domestic pigeons belong to family Columbidae are domesticated by humans for food, recreation and medical purposes (Mushtaq et al., 2006, Li et al., 2013). Pigeons are susceptible to a variety of infections, the most serious of which is Newcastle disease (Wang et al., 2017) Umar *et al*., 2015). This disease is highly infectious in pigeons and locally known as 'Jholah' in Punjab, Pakistan (Kapczynski et al., 2013, Akhtar et al., 2016). Avian paramyxovirus serotype-1 (APMV-1) in its velogenic form is endemic in the Middle East, Asia, Africa, Central America, and South America (Dimitrov et al., 2016, Wang et al., 2017). The virus can last in the environment for an extended period of time and propagate through direct contact with respiratory secretions, drooping, and contaminated items, including footwear, clothing, and equipment (Ellakany et al., 2019). The likelihood of epidemics is increased by the prohibited trade in exotic birds.

The disease is characterized by respiratory symptoms (gasping, coughing sneezing and rales), nervous indications (head tremor, torticollis, blurred vision, paralysis) and poor feathering (Marlier and Vindevogel, 2006). However, in acute cases, pigeons may develop green watery diarrhoea, dehydration, and chronic enteritis (Shaheen et al., 2005). Typically, the gross pathology lacks specificity, but congestion affects visceral organs (pancreas, kidneys, liver, spleen and thymus) and meninges (Chowdhary et al., 2020) . The incubation period is six to ten days in infants whereas three to four weeks in adults (Catroxo et al., 2011).

In 2011, Pakistan encountered its first occurrence of virulent NDV strains (Miller et al., 2015). The previous molecular studies on NDV reported prevalence of 41.3% (Aziz et al., 2023) in Pakistan, but no information is available regarding the epidemiology of Newcastle disease in Rawalpindi, Pakistan. This is the first study to investigate the prevalence, haemato-biochemical profile and histopathological, changes in naturally infected Newcastle disease virus pigeon in Rawalpindi, Pakistan.

## Materials and Methods

### Sample collection

The current study was conducted in 142 pigeon lofts in District Rawalpindi (42 lofts) and Gujar khan (100 lofts), Punjab, Pakistan, between September and July. A predesigned form was used to acquire the diseased related epidemiological information. A total of 50 clinically infected and 5 control group pigeons were slaughtered and 3 ml blood was collected for hematological and serum analysis, respectively.

### Haemato-biochemical Analysis

Complete blood count (CBC) of infected and normal pigeons was carried out to determined various hematological parameters such as red blood cells count, total leukocyte count, packed cell volume, hemoglobin, erythrocyte sedimentation rate, and mean corpuscular volume automated using haematology analyzer (Abbott Cell-Dyn 3700), Illinois USA).

Various biochemical parameters including serum total protein, albumin, alanine aminotransferase (ALT) and serum globulins were determined using AMP diagnostic kit on Metertek SP-8SO spectrophotometer (Korea), according to manufactures instruction.

### Histopathological and Immunology Studies

After slaughtering, several tissues (brain, intestine, and liver) were collected and fixed in 10% buffered formalin before embedding in paraffin wax. Subsequently, tissues were stained using eosin and hematoxylin, according to the protocols defined by (Bancroft and Gamble, 2008). The haem-agglutination inhibition assay was used for immunology examinations as described by OIE reference manual (2012).

### Statistical analysis

All the data were analyzed with Statistical package Minitab (version 16, USA) using one way ANOVA and Chi-Square test. The results are shown as Mean ± Standard deviation. The P value $< 0.05$ was considered as significant.

## Results

### Prevalence of Newcastle disease

The overall prevalence of ND was 18.45%, while it was highly significant in Gujar Khan 19.88% as compared to 14.68% in Rawalpindi and had 100% mortality (Table 1). The rural areas had significant infection rate 23.93%, as compared to urban areas 15.14% (Table 1). The prevalence of ND varied significantly among sampling seasons, with maximum in winter followed by summer, autumn and spring (Table 2). The incidence of infection was highest (90.40%) in loft of farmers that introduce new birds, whereas lowest (9.59%) in normal lofts (Table 3). When analyzed for purpose of keeping, the ND was highly significant (P=0.000) in domestic pigeons, as compared to racing, fancy and wild (Table 3). The vaccinated pigeons had significant reduced infection rate in contrast to non-vaccinated (Table 3). The clinical signs and pattern of



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diseases occurrence were also observed and showed significant variation among infected pigeons (Table 3).

**Associated Risk Factor**

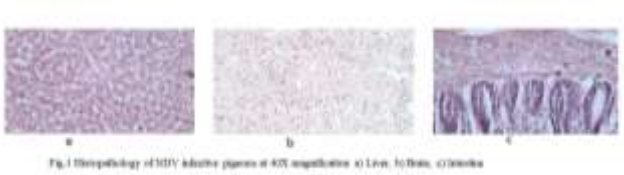
Furthermore, to investigate the impact of diseases on associated risk factors, blood chemistry, immunology, and histopathology, 50 (16 lofts) clinically infected pigeons were randomly collected and slaughtered. Analysis of data of the associated risk factors revealed that gender, age, sanitary conditions were non-significant ($p > 0.05$) with the incidence of disease (Table 4).

**Haemato-biochemical profile**

Comparison of the hematological parameters between clinically infected pigeons and control group revealed that there was significant increase in lymphocyte count and erythrocyte sedimentation rate, while drastically decreased in the hetrophil (Table 5). All other studied parameters varied non-significantly ($P > 0.05$) between the two groups. Moreover, serum biochemical profile and antibody titer of Newcastle disease virus remained non-significant when compared between clinically infected pigeons and control group (Table 6).

**Histopathology**

After slaughtering, the histopathological changes were observed in brain, liver and intestine of the pigeons naturally affected with ND virus. The analysis exhibited non-suppurative inflammatory changes with mononuclear cells infiltration in centro-lobular areas in brain (Fig. 1a), while evident hydropic degeneration and fatty changes in liver (Fig. 1b). The intestinal villi were misshapen, vessels in the lamina propria were dilated and cellular lining of the crypts of Lieberkuhn were hypertrophied in affected birds (Fig. 1c).



## Discussion

Pigeons are traditionally reared as a source of food, recreation, and recently their farming gained popularity worldwide. PPMV-1 are variants of NDV associated with infections of feral and domestic pigeons. In present study, the overall prevalence of ND was 18.45%. The previous studies on ND reported prevalence of 41.3% in Pakistan, (Aziz et al., 2023) 47.3 % Bangladesh (Islam et al., 2020) and 43.3% in Taiwan (Tsai and Lee, 2006). This higher incidence may be due to the close contact between pigeons and other poultry flocks (Abdelaziz et al., 2019)

The hematology profile of infected pigeons showed significant increase in lymphocyte count that may be attributed to the large flock sizes common in rural areas, as noted by (Abed, 2013). Similarly, elevated erythrocyte sedimentation, observed in clinically infected pigeons, is considered a hallmark of severe Newcastle Disease (ND) infection in pigeons. These findings are consistent with those reported by (Orakpoghenor et al., 2021, Oznurlu et al., 2012). In contrast, the significant decrease in heterophil count (avian equivalent of mammalian neutrophils) in infected pigeons, indicates severe systemic infections, as described by (Clark et al., 2009).

The biochemical profile specifically for alanine aminotransferase (ALT), showed non-significant increase in the infected group that may be attributed to hepatocyte damage caused by the paramyxovirus. The similar findings were described by Mushtaq et al. (2006) and (Ul-Rahman et al., 2020).

The histopathology of liver exhibited mononuclear cell infiltration and hepatic lesion in infected birds, suggesting an inflammatory response to the viral infection, consisted with findings by (Chowdhary et al., 2020). The examination of the brain tissue in pigeons infected with ND revealed numerous neuropathological changes including non-purulent meningo-encephalitis. Similar finding were reported in pigeons experimentally infected with paramyxovirus by (Al-Mubarak et al., 2020) and (Shaheen et al., 2005). Moreover, investigation of the intestinal tissue in ND-infected pigeons may reveal pathological changes included epithelial cell necrosis (Mudassir et al., 2014).

The research findings indicates the effectiveness of associated risk factor in the incidence and prevention of the disease and highlight significant haemato-biochemical and histopathological changes in infected birds.

## Conclusion

The study contributes in understanding the prevalence, risk factors, and pathological changes associated with ND to the development of a sustainable epidemiological approach to combat NDV in pigeons. Future research should focus on exploring more effective control measures and vaccination strategies to protect both domestic and wild pigeon populations.

## Author's contributions

MQ, designed this study. IAK participated in its coordination. SA and QUA participated in sample collection performed the experiments, and conducted data analysis. MA drafted and revised the manuscript.



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N/A.

**Conflict of interest**
The authors declare no conflict of interest with anyone.

**Ethical statement**
All the procedures for animal handling and lab protocols were approved by the ethical committee of Department of Zoology, Pir Mehr Ali Shah (PMAS) Arid Agriculture University Rawalpindi, Pakistan.

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**Table 1.** Prevalence of NDV in different localities

| Area | Number of birds | Number of infected birds | Percentage of infection | Chi-Sq. $\chi^2$ | P-value |
|---|---|---|---|---|---|
| Rawalpindi | 2880 | 423 | 14.68%b | 26.282 | 0.000*** |
| Gujar Khan | 7575 | 1506 | 19.88%a | | |
| Overall | 10455 | 1929 | 18.45 | | |
| Rural | 52 | 942 | 23.93% | 84.833 | 0.000*** |
| Urban | 90 | 987 | 15.14% | | |

P < 0.01 =Significant (**)

**Table 2.** Prevalence of NDV infection in different seasons

| Season | Diseased birds | Percentage of infected birds | Chi-Sq. $\chi^2$ | P-value |
|---|---|---|---|---|
| Spring | 150 | 7.77% | | |
| Summer | 589 | 30.53% | 487.730 | 0.000*** |
| Winter | 693 | 35.92% | | |
| Autumn | 281 | 14.56% | | |
| Not Defined | 216 | 11.19% | | |

P < 0.01 =SIGNIFICANT (**)



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**Table 3.** Associated risk factor in NDV disease

| Parameters | | Number of loft | Number of infected bird | Percentage of infection | Chi-Sq. $\chi^2$ | P-value |
|---|---|---|---|---|---|---|
| Introduction of birds | Yes | 120 | 1744 | 90.40% | 905.711 | 0.000*** |
| | No | 22 | 185 | 9. 59% | | |
| | Fancy | 19 | 721 | 114 | | |
| Purpose of keeping | Racing | 29 | 2737 | 555 | 70.550 | 0.000*** |
| | Wild | 5 | 216 | 20 | | |
| | Domestic | 64 | 4072 | 883 | | |
| Vaccination | Yes | 40 | 743 | 38.51% | 68.224 | 0.000*** |
| | No | 102 | 1186 | 61.48% | | |
| Diseases Occurrence | Sudden | 1929 | 1281 | 66.40% | 140.156 | 0.000*** |
| | Slow Onset | 1929 | 648 | 33.59% | | |
| Clinical signs | Respiratory | | 203 | 10.52%c | 937.179 | 0.000*** |
| | Nervous | | 1077 | 55.83%a | | |
| | Digestive | | 562 | 29.13%b | | |

P < 0.01 =Significant (**)



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**Table 4.** NDV infection at different gender, ages group and sanitary conditions (n = 50)

| Parameters | | Number of birds | Percentage o | Chi-Sq. $\chi^2$ | P-Value |
|---|---|---|---|---|---|
| Gender | Female | 25 | 50% | 0.000 | 1.000 |
| | Male | 25 | 50% | | |
| Age | Chick | 16 | 32% | 1.454 | 0.483 |
| | Sub adult | 13 | 26% | | |
| | Adult | 21 | 42% | | |
| Sanitary Conditions | Good | 17 | 34% | 2.769 | 0.250 |
| | Moderate | 11 | 22% | | |
| | Poor | 22 | 44% | | |

P < 0.01 =Significant (**)



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**Table 5.** Haematological analysis (Mean± SD) in NDV positive and negative pigeons**.**

| **Parameters** | **NDV** | | **P-value** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| PCV (%) | 48.705± 5.630 | 52.250± 1.909 | 0.086 |
| Hb Conc. (g/dl) | 11.648± 2.209 | 12.250± 0.463 | 0.449 |
| ESR (mm/h) | 1.9091±0.6404* | 0.5000±0.0000 | 0.000 |
| TEC (million/cmm) | 3.3000±0.9283 | 3.5750±0.1389 | 0.411 |
| TLC (/cmm) | 10680± 3396 | 13500± 1773 | 0.027 |
| Hetrophils% | 40.818± 5.244* | 50.750± 0.886 | 0.000 |
| Basophils% | 0.7955± 0.9543 | 0.5000± 0.5345 | 0.401 |
| Eosinophils% | 1.773± 1.395 | 1.000± 0.756 | 0.135 |
| Lymphocyte% | 51.750± 6.892* | 41.750± 1.909 | 0.000 |
| Monocyte% | 5.114± 2.895 | 6.000± 1.690 | 0.407 |

P < 0.01 =Significant (**)

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**Table 6.** Antibody titer and Serum analysis in NDV positive and negative pigeons

| **Parameters** | **NDV** | | **P-value** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Antibody titer | 70.91± 38.64 | 48.00± 17.10 | 0.108 |
| Serum profile | | | |
| Total protein | 3.3259±0.7252 | 3.425± 0.994 | 0.809 |
| Albumin | 2.0041± 0.2654 | 1.8400±0.4836 | 0.309 |
| Globulin | 1.3219±0.7497 | 1.5850±1.3992 | 0.563 |
| ALT | 120.29±71.48 | 78.25 ± 19.48 | 0.116 |



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