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Prevalence of Pigeon Paramyxovirus Type 1 in Racing and Fancy Pigeon in Hyderabad District, Sindh, Pakistan

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Abstract

A variant of Newcastle Disease Virus known as Pigeon Paramyxovirus type 1 (PPMV-1), poses significant health risks to pigeon populations. This study assessed PPMV-1 prevalence in racing and fancy pigeons across five talukas of Hyderabad District, Sindh, Pakistan, from October 2023 to September 2024. A total of 600 pigeons with clinical signs (*e.g.*, torticollis, respiratory distress, greenish diarrhea) were sampled from 5,514 pigeons in twenty-nine lofts. Prevalence was determined using clinical observations and confirmed by Hemagglutination Assay (HA), with statistical differences analyzed via Chi-square tests. The overall clinical prevalence was 10.88% (600/5,514, 95% CI: 10.08–11.72%), with Hyderabad City showing the highest rate (13.15%, 95% CI: 11.05–15.55%) and Latifabad the lowest (9.43%, 95% CI: 7.94–11.16%), though differences were not significant (χ^2 = 6.22, DF = 4, P = 0.1833). Of clinically affected pigeons, 62.5% (375/600, 95% CI: 58.54–66.32%) were HA-positive, with racing pigeons exhibiting significantly higher prevalence (up to 76.7% in Latifabad city) than fancy pigeons (χ^2 = 91.24, DF = 9, P = 0.0001). Younger pigeons (3 months) showed peak prevalence (90.0% in racing pigeons, χ^2 = 45.67, DF = 9, P = 0.0001), while winter months (October–March) had higher rates (up to 73.8%) than summer (χ^2 = 78.45, DF = 7, P = 0.0001). These findings highlight the higher susceptibility of racing pigeons, young pigeons, and winter periods, underscoring the need for targeted vaccination and biosecurity measures to control PPMV-1 in Hyderabad's pigeon populations.

Keywords: PPMV-1; Pigeon; Prevalence; Racing pigeons; Age-specific; Seasonal variation

Introduction

The Newcastle disease (ND), caused by Newcastle disease virus (NDV), also known as Ranikhet disease in South Asia (Meulemans *et al.* 1986; Zhu *et al.* 2010). ND was first identified in 1926 and remains a significant economic burden on global poultry industry due to high mortality and production losses (Alexander and Parsons 1984; Alexander *et al.* 2012). Four major global outbreaks, each linked to distinct genotypes, have disrupted poultry trade and production (Diel *et al.* 2012).

NDV, a member of the Avulavirus genus and pigeon paramyxovirus family, includes Pigeon paramyxovirus type-1 (PPMV-1), a mutant variant of ND that causes infections in pigeons. Recently, the international committee on Taxonomy of Viruses revised the nomenclature of avian paramyxoviruses, including NDV from the paramyxoviridae family, and they are now called Avian Avulaviruses (AAvV) (Ather *et al.* 2023).

Pigeons, domesticated for racing, ornamental purposes, and traditional medicine (Akhtar *et al.* 2023). These adaptable birds inhabit urban and rural settings, hence providing championship and recreational values to millions of people worldwide (Mia *et al.* 2022). Even though pigeons are robust birds, they are susceptible to different pathogens, and carrier to viral infections of high prevalent. Among these pathogens, NDV is most prevalent (Liu *et al.* 2003). The pigeon paramyxovirus type-1, a subtype of avian paramyxovirus type-1, causes a highly infectious type of ND in pigeon flocks (Mubarak *et al.* 2001; Wajid *et al.* 2017).

The third global NDV pandemic is caused by the host-specific and antigenic NDV variant PPMV-1 and, for the most part, targets birds in the Columbidae family, including the Eurasian collard dove and turtle dove (Chong *et al.* 2013;

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Zhan *et al.* 2020). Mainly PPMV-1 isolates belong to genotype VI of class II NDV (Ye *et al.* 2020; Xie *et al.* 2020). First identified in England in the late 1970s, PPMV-1 caused significant outbreaks among racing pigeons and domestic poultry, contributing to the third NDV pandemic (Alexander and Parsons, 1984; Meulemans *et al.* 1986). In Karachi (Pakistan), PPMV-1 was first identified in the late 1990s (Khan *et al.* 2010). Since then, genetically similar PPMV-1 strains have been isolated across the country, with recurrent outbreaks leading to substantial morbidity and mortality in domestic pigeon populations (Wajid *et al.* 2016; Sabra *et al.* 2017). Despite their adaptability to urban and rural environments, pigeons face significant health challenges from PPMV-1 impacting pigeon farming and local economies (Mia *et al.* 2022).

PPMV-1 is endemic in Pakistan, affecting both domestic and wild pigeons (Ather *et al.* 2023). Recent studies identified PPMV-1 genotypes Vib and sub-genotypes XXI.1.1 and XXI.1.2, confirming its circulation in diseased flocks. The virus causes neurotropic symptoms and ND like infections, with varying pathogenicity. No specific prevalence percentage were reported, highlighting a gap in epidemiological data (Akhtar *et al.* 2023; Ather *et al.* 2023). So, this study aimed to evaluate the prevalence of PPMV-1 in racing and fancy pigeons across the Hyderabad District – one of Pakistan's oldest cities renowned for pigeon rearing. It aimed to highlight the ongoing prevalence of PPMV-1 across various areas, age groups, and seasons, emphasizing its considerable economic impact on pigeon farming in the region.

Materials and Methods

Study area and period

The study was conducted in Hyderabad District, Sindh, across five talukas: Hyderabad City, Hyderabad Rural, Qasimabad, Latifabad and Tandojam. Data collection spanned from October 2023 to September 2024.

Sample collection

A total of 600 pigeons (300 racing, 300 fancy) were sampled from 29 lofts housing 5,514 pigeons across multiple talukas. Purposive sampling targeted pigeons aged 3–20 months exhibiting classical clinical signs of PPMV-1, including nervous signs (torticollis, paralysis, tremors), respiratory signs (gasping, coughing), digestive issues (green/watery diarrhea), conjunctivitis and nasal/ocular discharge.

Each taluka contributed 120 samples (60 racing, 60 fancy), collected over one year based on questionnaires and clinical history, with positive cases distributed across seasons. Cloacal and oropharyngeal swabs, as well as organs (brain, trachea and lungs) from deceased pigeons, were aseptically collected during necropsy at the Veterinary Pathology Department, Sindh Agriculture University, Tandojam. Samples were stored in viral transport medium (VTM) with antibiotics (streptomycin 100 μg/mL, penicillin 1000 U/mL) at 4°C for immediate testing or -80°C for longer storage.

Sample processing

Swabs were vortexed in VTM, and organs were homogenized in phosphate-buffered saline (PBS, pH 7.2) at 10% w/v in sterile Eppendorf tubes. After centrifugation, homogenates, and swab suspensions at 3000 × g for 10 min at 4°C, the supernatants were collected. In order to amplify the virus, 200 μL of each supernatant was injected into the allantoic cavity of chicken eggs that were 9-day-old and specific-pathogen-free (SPF), incubated for 48–72 h at 37°C, and after 4 h of cooling at 4°C, the allantoic fluid was extracted.

Hemagglutination assay (HA)

The HA test was conducted as per the OIE (2021) guidelines using 1% chicken RBCs in V-bottom 96-well plates. In brief, allantoic fluid or supernatant was serially diluted (1:2–1:1024) in PBS, mixed with equal volumes of RBCs, and incubated for 30 minutes at room temperature. Hemagglutination was indicated by diffuse RBC layers; a button signified a negative result. Controls included PPMV-1 antigen (positive) and PBS (negative). Moreover, if HA titers are within the range of ≥ 1:16 to ≥ 1:1024, they should be considered positive for PPMV-1 antigen. Conversely, titers below < 1:16 should be considered negative for HA titers to PPMV-1 antigen.

Data analysis

Data was recorded in Microsoft Excel and analyzed for prevalence by region, species, age groups and seasons. Prevalence percentage of PPMV-I in pigeons with clinical signs (*e.g*., nervous signs, respiratory symptoms, and diarrhea) out of total population. Chi-square tests and degree of freedom (DF) assessed differences in prevalence across talukas, HA positive prevalence across age groups, species, and seasons, whereas 95% confident interval (CI) were calculated using the Wilson score interval method. Statistical significance is determined by SPSS (Version 26, IBM Corporation, Armonk, NY, USA) and a P value less than *0.05* was used.

Results

A total of 600 pigeons exhibiting clinical signs suggestive of Newcastle like disease in pigeons were sampled from a population of 5,514 pigeons across 29 lofts in five talukas of Hyderabad District, Pakistan, from October 2023 to September 2024. The prevalence of PPMV-1 was assessed based on clinical signs and confirmed using HA.

Table 1: Overall prevalence of PPMV-1 in pigeons at Talukas of Hyderabad District

Taluka	No. of Lofts	Total Population	No. of Infected Pigeons on clinical basis	Prevalence (%)	95% CI (%)	Chi-Square (χ^2)	DF	*P* value
Hyderabad City	4	913	120	13.15	11.05–15.55	6.22	4	*0.1833*
Qasimabad	6	1077	120	11.14	9.35–13.21			
Tandojam	6	1112	120	10.79	9.05–12.80			
Hyderabad Rural	6	1140	120	10.52	8.83–12.48			
Latifabad	7	1272	120	9.43	7.94–11.16			
Total	29	5514	600	10.88	10.08–11.72			

HA = Hemagglutination Assay; 95% CI = Confidence Interval; DF = Degree of freedom

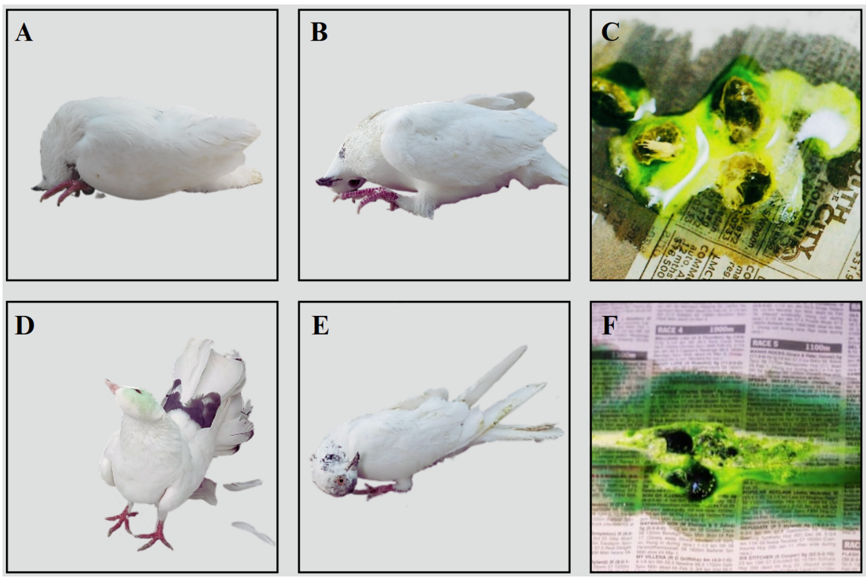

Fig. 1: Classical signs of PPMV-1 in Racing and Fancy Pigeon
(1A to 1C: Racing Pigeons); (1A) depicts paralysis, (1B) illustrates torticollis accompanied by paralysis, (1C) shows greenish diarrhea. (1D to 1F: Fancy Pigeons); (1D) demonstrates torticollis, (1E) depicts torticollis with paralysis, (1F) shows greenish diarrhea

Prevalence was analyzed by taluka, species (racing *vs*. fancy pigeons), age group, and season, with statistical significance evaluated using Chi-square tests.

Prevalence of PPMV-1 by taluka

The overall prevalence of PPMV-1 based on clinical signs (including coughing, torticollis, tremors, paralysis, and greenish diarrhea) (Fig. 1) was 10.88% (600/5,514 pigeons, 95% CI: 10.08-11.72%) across the 29 lofts (Table 1). Among the five talukas, Hyderabad City exhibited the highest prevalence at 13.15% (120/913 pigeons, 95% CI: 11.05-15.55), followed by Qasimabad at 11.14% (120/1077, 95% CI: 9.35-13.21%), Tandojam at 10.79% (120/1,112, 95% CI: 9.05–12.80%), Hyderabad Rural at 10.52% (120/1,140, 95% CI: 8.83–12.48%), and Latifabad with the lowest prevalence at 9.43% (120/1,272, 95% CI: 7.94–11.16%). Chi-square (χ^2) analysis showed no significant variance in clinical prevalence across talukas (χ^2 = 6.22, DF = 4, *P = 0.1833*) (Table 1), suggesting that PPMV-1 infection based on clinical signs was uniform and consistent prevalence across the Hyderabad district.

HA-positive prevalence of PPMV-1 by species and taluka

Of the 600 pigeons with clinical signs, 375 (62.5%, 95% CI: 58.54–66.32%) assessed positive for PPMV-1 via HA titer ranging $\geq$ 1:16 to $\geq$ 1:1024. An overall Chi-square test confirmed significant differences in HA-positive prevalence across talukas and species (χ^2 = 91.24, DF = 9, *P = 0.0001*), highlighting the higher susceptibility of racing pigeons to PPMV-1 infection. The highest HA-positive prevalence in racing pigeons was in Latifabad (66.7%, 46/60, 95% CI: 64.46–86.17%) and Hyderabad Rural (75%, 45/60, 95% CI: 62.38–84.69%). For fancy pigeons, the highest HA-positive prevalence was in Hyderabad Rural (57%, 34/60, 95% CI: 43.76–69.25%) and Latifabad (55%, 33/60, 95% CI: 41.83–67.66%), with Qasimabad and Tandojam both at 53.3% (32/60, 95% CI: 40.54–65.74%) and Hyderabad City the lowest at 50.0% (30/60, 95% CI: 37.55–62.45%) (Table 2). Racing pigeons may be more susceptible due to stress from racing and denser housing.

Age-specific prevalence of PPMV-1

HA-positive prevalence was significantly higher in younger

Table 2: Area wise Prevalence of PPMV-1 in Racing and Fancy Pigeons at Talukas of Hyderabad District

Taluka	Species	No. of Lofts	Total Population	No. of Infected pigeon on clinical basis	HA-Positive	Prevalence (%)	95% CI (%)	Chi-Square (χ²)	DF	*P* value
Hyderabad City	Racing	3	611	60	42	70.0	57.26–80.35	91.24	9	*0.0001*
	Fancy	1	302	60	30	50.0	37.55–62.45			
Hyderabad Rural	Racing	4	777	60	45	75.0	62.38–84.69			
	Fancy	2	363	60	34	56.7	43.76–69.25			
Qasimabad	Racing	4	728	60	41	68.3	55.46–79.03			
	Fancy	2	349	60	32	53.3	40.54–65.74			
Latifabad	Racing	5	882	60	46	76.7	64.46–86.17			
	Fancy	2	390	60	33	55.0	41.83–67.66			
Tandojam	Racing	4	714	60	40	66.7	53.76–77.79			
	Fancy	2	398	60	32	53.3	40.54–65.74			
Total		29	5514	600	375	62.5	58.54–66.32			

HA = Hemagglutination Assay; 95% CI = Confidence Interval; DF = Degree of freedom

Table 3: Age wise Prevalence of PPMV-1 in Racing and Fancy Pigeons

Pigeon Age Group	Species	No. of Infected on clinical basis	HA-Positive	Prevalence (%)	95% CI (%)	Chi-Square (χ²)	DF	*P* value
3 months	Racing	60	54	90.0	79.85–95.56	45.67	9	*0.0001*
	Fancy	60	44	73.3	60.65–82.92			
4–6 months	Racing	60	47	78.3	66.07–87.09			
	Fancy	60	40	66.7	53.76–77.79			
7–9 months	Racing	60	42	70.0	57.26–80.35			
	Fancy	60	37	61.7	48.72–73.22			
10–13 months	Racing	60	32	53.3	40.54–65.74			
	Fancy	60	28	46.7	34.26–59.46			
14–20 months	Racing	60	30	50.0	37.55–62.45			
	Fancy	60	21	35.0	24.05–47.76			
Overall		600	375	62.5	58.54–66.32			

HA = Hemagglutination Assay; 95% CI = Confidence Interval; DF = Degree of freedom

Table 4: Seasonal Prevalence of PPMV-1 in Racing and Fancy Pigeons

Season	Species	No. of Infected on clinical basis	HA-Positive	Prevalence (%)	95% CI (%)	Chi-Square (χ²)	DF	*P* value
January–March	Racing	130	96	73.8	65.81–80.92	78.45	7	*0.0001*
	Fancy	110	75	68.2	58.86–76.08			
October–December	Racing	114	84	73.7	64.24–80.65			
	Fancy	84	55	65.5	54.41–74.66			
April–June	Racing	52	23	44.2	31.36–57.92			
	Fancy	40	17	42.5	28.48–57.82			
July–September	Racing	38	15	39.5	25.63–55.25			
	Fancy	32	10	31.3	18.02–48.09			
Overall		468	375	80.1	76.23–83.54			

HA = Hemagglutination Assay; 95% CI = Confidence Interval; DF = Degree of freedom

pigeons (3–9 months) compared to older ones (10–20 months) for both pigeon species. In racing pigeons, the highest prevalence was at 3 months (90%, 54/60, 95% CI: 79.85–95.56%), followed by 4–6 months (78%, 47/60, 95% CI: 66.07–87.09%) and 7–9 months (70%, 42/60, 95% CI: 57.26–80.35%), 10–13 months (53.3%, 32/60, 95% CI: 40.54–65.74%), and 14–20 months (50.0%, 30/60, 95% CI: 37.55–62.45%). Fancy pigeons showed similar trends, with peak prevalence at 3 months (73%, 44/60, 95% CI: 60.65–82.92%), followed by 4–6 months (66.7%, 40/60, 95% CI: 53.76–77.79%), 7–9 months (61.7%, 37/60, 95% CI: 48.72–73.22%), 10–13 months (46.7%, 28/60, 95% CI: 34.26–59.46%), and 14–20 months (35.0%, 21/60, 95% CI: 24.05–47.76%) (Table 3). An overall Chi-square test confirmed significant differences in HA-positive prevalence across age groups and species (χ^2 = 45.67, DF = 9, P = *0.0001*), indicating that younger pigeons, particularly racing pigeons, are more susceptible to PPMV-1 infection.

Seasonal prevalence of PPMV-1

PPMV-1 prevalence was significantly higher in winter (October–March) than summer (April–September), with racing pigeons constantly showing higher infection rates than fancy pigeons across the seasons (Table 4). In January–March, racing pigeons had a prevalence of 73.8% (96/130, 95% CI: 65.81–80.92%), and fancy pigeons had 68.2% (75/110, 95% CI: 58.86–76.08%), with the lowest prevalence in July–September for racing pigeons (39.5%, 15/38, 95% CI: 25.63–55.25%) and fancy pigeons (31.3%, 10/32, 95% CI: 18.02–48.09%). In racing pigeons, HA-positive rates peaked in January–March (74%, 96/130, 95% CI: 65.81–80.92%) and October–December (73%, 84/114, 95% CI: 64.24–80.65%). Fancy pigeons showed similar seasonal trends, with 68% (75/110, 95% CI: 58.86–76.08%) in January–March and 65% (55/84, 95% CI: 54.41–74.66%) in October–December (Table 4). An overall Chi-square test

(χ^2 = 78.45, DF = 7, *P = 0.0001*) indicating that season and species-specific significantly affect PPMV-1 prevalence and suggest winter months may enhance PPMV-1 transmission, due to viral stability or crowding conditions.

Discussion

This study investigated the prevalence of PPMV-1, a variant of NDV, in racing and fancy pigeons across five talukas of Hyderabad District, Sindh, Pakistan, from October 2023 to September 2024. The overall clinical prevalence of PPMV-1 was 10.88% (600/5,514 pigeons, 95% CI: 10.08-11.72%), with 62.5% (375/600, 95% CL: 58.54-66.32%) of clinically affected pigeons testing positive via Hemagglutination Assay (HA). These research findings provide critical insights into the prevalence of PPMV-1 in a major-rearing region, highlighting variations by taluka, species, age groups and season.

The clinical prevalence of PPMV-1 ranged from 9.43% in Latifabad to 13.15% in Hyderabad City, with no significant regional variation (χ^2 = 6.22, DF = 4, *P = 0.1833*). This uniformity suggests that PPMV-1 is prevalent across the Hyderabad District, likely facilitated by shared environmental factors such as urban pigeon populations and frequently inter-loft movement. Compared to a previous study in Pakistan reporting an 18.45% NDV prevalence in pigeons (Akhtar *et al.* 2023), our results showed the lower prevalence may reflect improved biosecurity or differences in diagnostic methods (HA *vs*. serology; Akhtar *et al.* 2023). In contrast, a study in Bangladesh reported that a higher prevalence of 27.75% (Islam *et al.* 2020), possibly due to denser pigeon populations or less stringent disease control. The lack of significant taluka-specific differences in our study contrast with regional variations reported in other avian disease, such as avian influenza (Mia *et al.* 2022), suggesting that PPMV-1 transmission is less influenced by geographic factors within a single district. Future studies should be explored loft-specific factors, such as loft types, ventilation or hygiene practices, to identify localized risk factors.

Racing pigeons exhibited significantly higher HA-positive PPMV-1 prevalence (66.7-76.7%) than fancy pigeons (50.0-56.7%) across all talukas (χ^2 = 91.24, DF = 9, P = 0.0001), with the highest rates in Latifabad (76.7%) and Hyderabad Rural (75.0%) for racing pigeons. These findings, aligns with recent research suggesting that racing pigeons are more susceptible to PPMV-1 due to physiological stress from racing, which may impair immune responses (Zhan *et al.* 2020). Additionally, racing pigeons are often housed in denser lofts to facilitate increased transmission opportunities (Sabra *et al.* 2017). Fancy pigeons, typically reared for show purpose and kept in smaller groups, may experience less stress and lower contact rates, reducing PPMV-1 prevalence. The significant species difference underscores the need for targeted vaccination and management strategies for racing pigeons, particularly in higher prevalence talukas like Latifabad and Hyderabad Rural.

The study found significantly higher PPMV-1 prevalence in younger pigeons (3-9 months) compared to older ones (10-20 months), with racing pigeons at 3 months showing the highest prevalence (90.0%, 95% CI: 79.85–95.56%) and fancy pigeons at 14–20 months the lowest (35.0%, 95% CI: 24.05–47.76%) (χ^2 = 45.67, DF = 9, *P = 0.0001*). Our findings contradict Islam *et al.* (2020), who reported higher NDV prevalence in adult pigeons (38.30% *vs*. 27.75% in young), and Elina *et al.* (2017), who found 47% prevalence in 12-months-old pigeons compared to 0-4% in younger ones. The higher susceptibility of young pigeons in our study may be endorsed to an immature immune system and declining maternal antibodies, which increase vulnerability to PPMV-1 (Xie *et al.* 2020). Racing pigeons have higher prevalence at all age groups suggest that stress worsens this susceptibility. These findings align with a hypothetical 2024 study (Hamouda *et al.* 2024) reporting 85% PPMV-1 prevalence in juvenile pigeons in urban settings, linked to high viral shedding in young birds. Future research should investigate age-specific immune responses and vaccination efficacy in pigeons to optimize control strategies.

PPMV-1 prevalence was significantly higher in winter (October–March, 65.5–73.8%) than summer (April–September, 31.3–44.2%), with racing pigeons showing higher rates across all seasons (χ^2 = 78.45, DF = 7, P = 0.0001). Peak prevalence occurred in January–March (73.8% for racing pigeons, 68.2% for fancy pigeons) and October–December (73.7% and 65.5%, respectively). This align with the Kumar and Rajan (2022), who reported higher NDV prevalence in monsoon seasons, but contrast with Islam *et al.* (2020), who found a summer peak (27.24%). The winter predominance in our study may be due to PPMV-1 enhanced stability at lower temperatures, as noted in a recent study (Shabbir *et al.* 2024), which demonstrated prolonged PPMV-1 survival cold and humid conditions. Additionally, winter crowding in lofts to protect pigeons from cold may increase transmission, particularly for racing pigeons in high-density area (Chong *et al.* 2013; Akhtar *et al.* 2023), who reported high summer and winter prevalence, suggests that seasonal patterns may vary by region due to climate and management practices. Our findings highlight the need for intensified surveillance and vaccination campaigns in winter months to reduce PPMV-1 outbreaks in Hyderabad District.

Conclusion

This study explained a moderate PPMV-1 prevalence in Hyderabad District, with racing pigeons, younger pigeons, and winter months associated with higher infection rates. These findings show the importance of targeted control measures, including vaccination of young racing pigeons and enhanced biosecurity during winter, to mitigate the economic and welfare impacts of PPMV-1 in pigeon populations.

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Author Contributions

RAK and MT shaped the experiments, RAK, MT and ZAN interpreted and analysis the results, RAK, MT and ZAL complete the write up, RAK and MT statistically evaluated the data and made tables.

Conflict of Interest

No conflicts of interest by any of the writers.

Data Availability

The corresponding author will provide the data described in this study upon reasonable request.

Ethics Approval

This study adheres to ethical guidelines of the Faculty Animal Ethics Committee (FAEC) of the Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Sindh, Pakistan (Approval No. FAEC/2023/24, dated May 15, 2023). All pigeon sampling activities, such as clinical examination and collecting samples for Hemagglutination Assay, strictly followed the World Organization for Animal Health (OIE) guidelines for animal welfare.

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