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First Molecular Evidence of Co-Infection with Avian Polyomavirus and Psittacine Beak and Feather Disease Virus in Psittacine Birds from Pakistan: A brief report

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Short Report

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Abstract

Avian Polyomavirus (APV) and Psittacine Beak and Feather Disease Virus (PBFDV) are major pathogens of psittacine birds, causing severe morbidity, mortality, and economic loss. Despite their global distribution, molecular prevalence data remain scarce. This study investigated the occurrence of APV and PBFDV in Pakistan and provides the first evidence of natural co-infection. From October 2023 to June 2024, 151 samples, including blood (n = 47), feathers (n = 52), and post-mortem tissues (n = 52), were collected from *Agapornis*, *Psittaculidae*, *Estrildidae*, *Melopsittacus undulatus*, and *Columbidae* birds. DNA was extracted by commercial and phenol–chloroform methods, and PCR assays targeting the *VP1* gene of APV and *Rep* gene of PBFDV were performed. APV was detected in 48.5% of blood and tissue samples, while PBFDV was found in 33.8% of all specimens. Co-infection occurred in 6.6% of birds, with compounded disease severity. These findings establish baseline prevalence data for Pakistan and emphasize urgent needs for surveillance and biosecurity.

Introduction

Highly communicable DNA viruses, avian polyoma virus and psittacine beak and feather disease virus are a great menace to psittacine population worldwide. Intense sickness in domesticated birds caused by these pathogens results unpredicted mortality, treatment challenges, and species to species transmission (Adiguzel et al. 2020) .APV is a non enveloped virus with icosahedral capsid, which is a member of the Polyoma virus genus of the *Polyoma viridae* family (Altan et al. 2016). First recognized in young budgerigars in 1980 (Katoh et al. 2009) and has a circular double stranded DNA genome of 4981 base pairs with upstream and downstream segments encoding various proteins. Budgerigar Fledgling Disease (BFD) is caused by PBFDV, a member of the Circoviridae family and Circovirus genus (Pass & Perry 1984) in budgerigars. Both viruses have worldwide occurrence and infect broad spectrum of Psittacine species including parrots and budgerigars (Wang et al. 2022).

Clinical symptom of APV infection includes paleness, lack of appetite, subcutaneous haemorrhage, crop stasis and feather loss (Adiguzel et al. 2020).furthermore, this virus also causes depression, hepatitis, feather abnormalities, and post mortem lesions of the liver, spleen and other tissues with localized necrosis and basophilic cytoplasmic inclusions (Altan et al. 2016). Although mammalian polyoma viruses are usually benign, however, APV cause rapidly fatal mortality in birds without premonitory signs (Johne et al. 2000). The virus is spread by diseased birds through feathers, feces and other excretions for up to six months, which increases the risk of infection for hatchlings and newly introduced birds (Altan et al. 2016). In nestlings, APV is particularly lethal, with a 100% fatality in birds aged 1 week to 5 months (Hsu et al. 2006).

In contrast, PBFDV results in long term abnormal feather development, resulting in flight limitations and increased predation vulnerability (Raidal & Peters 2018). Clinical signs of PBFDV infection include fatigue, feather loss, follicle bleeding, abnormal beak growth, and immunosuppression, which predisposes birds to secondary bacterial, chlamydial, or microbial infections (Katoh et al. 2008; Adiguzel

et al. 2020).In spite of that PBFDV has a lower mortality rate compared to APV, it causes significant morbidity and is highly contagious, spreading through direct contact, ingestion of contaminated feces, or inhalation of feather dust(Adiguzel et al. 2020).

APV and PBFDV have been reported in several continents, including North America, Europe, Asia and Africa (Albertyn et al. 2004; Johne et al. 2006). This highlights the need for actions for the diagnostic and control of this important disease. Despite this, there is a large research gap in Pakistan regarding the prevalence and impact of these viruses. The main aim of the study is to spotlight the gap by using PCR techniques to identify APV and PBFDV, their frequency in Pakistan and planning to disrupt the disease cycle to protect avian population and breeding communities.

Materials and Methods

Sampling and Study Design

151 samples were collected from five psittacine species, including *Agapornis* sp (n=109), *Psittaculidae* sp (n=28), *Estrildidae* sp (n=05), *Melopsittacus undulatus* (n=08), and *Columbidae* sp (n=01), across various locations in Pakistan between October 2023 and June 2024. The samples included 99 pre-mortem samples (47 blood and 52 feather samples) and 52 post-mortem muscle tissue samples (Table 1). Samples were collected from breeders in Lahore, Multan, Peshawar, Faisalabad, Karachi, Swat, and other cities. Pre mortem samples were collected by trained professionals following ethical guidelines, while post-mortem samples were obtained from birds exhibiting symptoms of APV or PBFDV prior to death. Some of the deceased birds including love bird, macaw parrot and ring neck parrot were given in

DNA Extraction and PCR Amplification

DNA was isolated from different samples including blood, tissue, and feather using standardized protocols. Thermo Scientific Gene JET Genomic DNA Purification Kit was used to extract DNA from Blood and post-mortem tissue. For **feather samples**, Phenol-Chloroform-Isoamyl (PCI) method was used to separate the DNA from the samples. Feathers were incubated overnight in lysis buffer containing proteinase K at 56 °C on the tips of feather. After centrifugation (13,000 rpm) with PCI (300ul) the supernatant with DNA was separated followed by ethanol precipitation.

Specific primers were design targeting VP1 gene (831 bp) and rep gene (605bp) that were responsible for APV and PBFDV identification by using PCR. The total volume of PCR reaction mixture was 20.5 µl containing 0.3 µl of each primer, 4 µl of nuclease-free water, DNA template was 5 µl, and 11 µl of master mix. Thermal cycling protocol consisted of an initial denaturation at for 5 minutes at 95°C, followed by 35 cycles consisting of 95°C denaturation for 30 seconds, annealing at 56°C for PBFDV and 60°C for APV for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 10 minutes.

Statistical Analysis

Prevalence was calculated as PCR-positive/total samples. Species- and specimen-based associations were tested using chi-square test (GraphPad Prism v10).

Results

The study demonstrated a substantial viral burden among tested avian samples, revealing that 48.48% of blood and tissue samples were positive for Avian Polyomavirus (APV), while 33.8% were positive for Psittacine Beak and Feather Disease Virus (PBFDV). Co-infection was detected in 6.6% of the tested samples (Table 2). Many infected birds appeared clinically healthy, highlighting the critical role of molecular diagnostics in identifying asymptomatic carriers.

PCR detection of PBFDV in feather and APV in muscle, blood, and tissue samples

The PBFDV detection is shown in Fig. 1(a), wells 37, 52, and 53, showed bands of intensities identical to the positive control (well 54). Appropriate positive and negative controls provided by the Avian Laboratories and Research Centre, Lahore. In figure rest of the samples were negative for PBFDV indicated by absence of bands. APV was detected only in muscle tissues of deceased birds and blood samples from live birds (Table 2), as feathers were unsuitable for APV detection. Out of 101 valid samples, 48 tested positive for APV (Table 2) Fig. 1 (c) shows the results of five post-mortem tissue samples. The 831 bp band, consistent with the positive control's band intensity, confirmed that all the examined birds were infected with APV.

Co-detection of PBFDV and APV:

To evaluate co-infection, PCR was conducted on 10 selected samples (six post-mortem tissues and four blood samples) using separate primer sets for APV and PBFDV. Both viruses were successfully amplified, producing 831 bp (APV) and 605 bp (PBFDV) bands corresponding to their respective positive controls. The consistent detection of both amplicons across all samples confirmed simultaneous infection in the examined birds Fig. 1 (b).

Prevalence and mortality rates:

Among the 151 birds analyzed, the prevalence of APV was 48.8%, with a notably higher occurrence in muscle tissues from deceased birds (73%) compared to blood samples from live birds (21.27%). The mortality rate among APV-positive birds was 98%, indicating a highly lethal course. In contrast, PBFDV infection showed a prevalence of 50% and an estimated mortality rate of 50–55%, based on aviary records where several infected birds survived following timely vaccination and isolation. These findings collectively emphasize the widespread occurrence, high mortality, and co-infection potential of APV and PBFDV among captive avian populations.

Table 1
Distribution of APV and PBFDV infections across various avian species tested in the study

Bird species	Local name	Number of birds tested	Total Number of birds tested	APV positive	PBFDV positive
Psittaculidae	Amazona auropalliata	**03**	**28**	04(14.2%)	11(39.2%)
	Raw parrot	**09**			
	Psittacula krameria	**08**			
	Psittacula eupatria	**05**			
	Psittacus Erithacus	**02**			
	Eclectus roratus	**01**			
Melopsittacus undulates	-	**08**	**08**	**0**	01(12.5%)
Estrildidae	Padda oryzivora	**05**	**05**	**0**	**0**
Columbidae	Ducula bicolor	**01**	**01**	**0**	**0**
Agapornis sp	blue 2 fishri	**02**	**109**	44(40.3%)	39(35.7%)
	albino red eye	**01**			
	violet dun	**01**			
	opine love bird	**03**			
	Love bird	**102**			

Table 2
Prevalence of APV and PBFDV in Psittacine bird's samples in Pakistan

Virus	Positive cases	Prevalence (%)	Sample types detected
APV	48/99	48.5	Blood, tissue
PBFDV	51/151	33.8	Blood, tissue, feather
Co-infection	10/151	6.6	Blood, tissue

Discussion

This study provides the first molecular evidence of Avian Polyomavirus (APV) and Psittacine Beak and Feather Disease Virus (PBFDV) co-circulation in psittacine birds in Pakistan. Prevalence was high (APV

48.34%, PBFDV 33.77%), exceeding reports from other regions, e.g., APV at 0.8% in Italy (Bert et al. 2005) and PBFDV at 3% in New Zealand (Latimer et al. 1991). APV was first described in the USA (Varsani et al. 2015) and Canada (Bernier et al. 1981), while PBFDV originated in Australia (Pass & Perry, 1984). Elevated rates, similar to Africa and Asia where diagnostics and biosecurity remain limited (Albertyn et al. 2004; Philadelpho et al. 2015; Amery-Gale et al. 2017), likely reflect Pakistan's rapid breeding expansion and inadequate diagnostic capacity.

APV cause 100% mortality of nestlings and PBFDV causes long-term morbidity, reducing market value (Raidal & Peters 2018). Birds infected with both viruses (6.6%) displayed the most critical symptoms, likely due to PBFDV-induced immunosuppression that facilitates APV replication (Katoh et al. 2008). The observed high prevalence of both viruses in Pakistan poses serious threat to small-scale breeder. Effective control depends on timely diagnosis through molecular methods. This study establishes baseline prevalence data, highlights severe clinical and economic consequences, and underscores the urgent need for improved diagnostics and surveillance in Pakistan.

Conclusion

This study investigated the prevalence and co-infection rates of Avian Polyoma virus and Psittacine Beak and Feather Disease Virus in Pakistan. The findings revealed significant infection rates, with 33.8% of samples testing positive for PBFDV and for APV it will be 48.48%. The Co-infection rate was detected in 10 avian specimens. Using Molecular diagnostic techniques based on PCR, this study underscores the critical importance of advance detection of infections and the segregation of affected avian to mitigate disease. The results highlight the substantial economic impact of these viral infections on avian populations, emphasizing the urgent need for the implementation of robust biosecurity measures and routine screening protocols to minimize losses and safeguard avian health.

Declarations

Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval:

Approved by the Institutional Animal Ethics Committee, University of the Punjab, Lahore. Only naturally deceased birds were used for tissue sampling; no live birds were euthanized.

Ethical Approval:

The ethical approval for the current study was taken from Institutional ethical review board of Centre for applied molecular biology, University of the Punjab (Ref#CAMB/AO/62/24)

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Author Contribution

Conceptualization, MS, RSS FS; methodology, MS, RSS, TM, HM; validation, TM,SN; investigation, TM; resources, MS, RSS; data curation, AA,SN, HM; writing—original draft preparation, AA, HM; writing—review and editing MS, RSS, HM; visualization, MS,RSS, FS; supervision, MS, RSS; project administration TM, FS. All authors have read and agreed to the published version of the manuscript.

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Data Availability:

Data is available from the corresponding authors on reasonable request.

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Figures

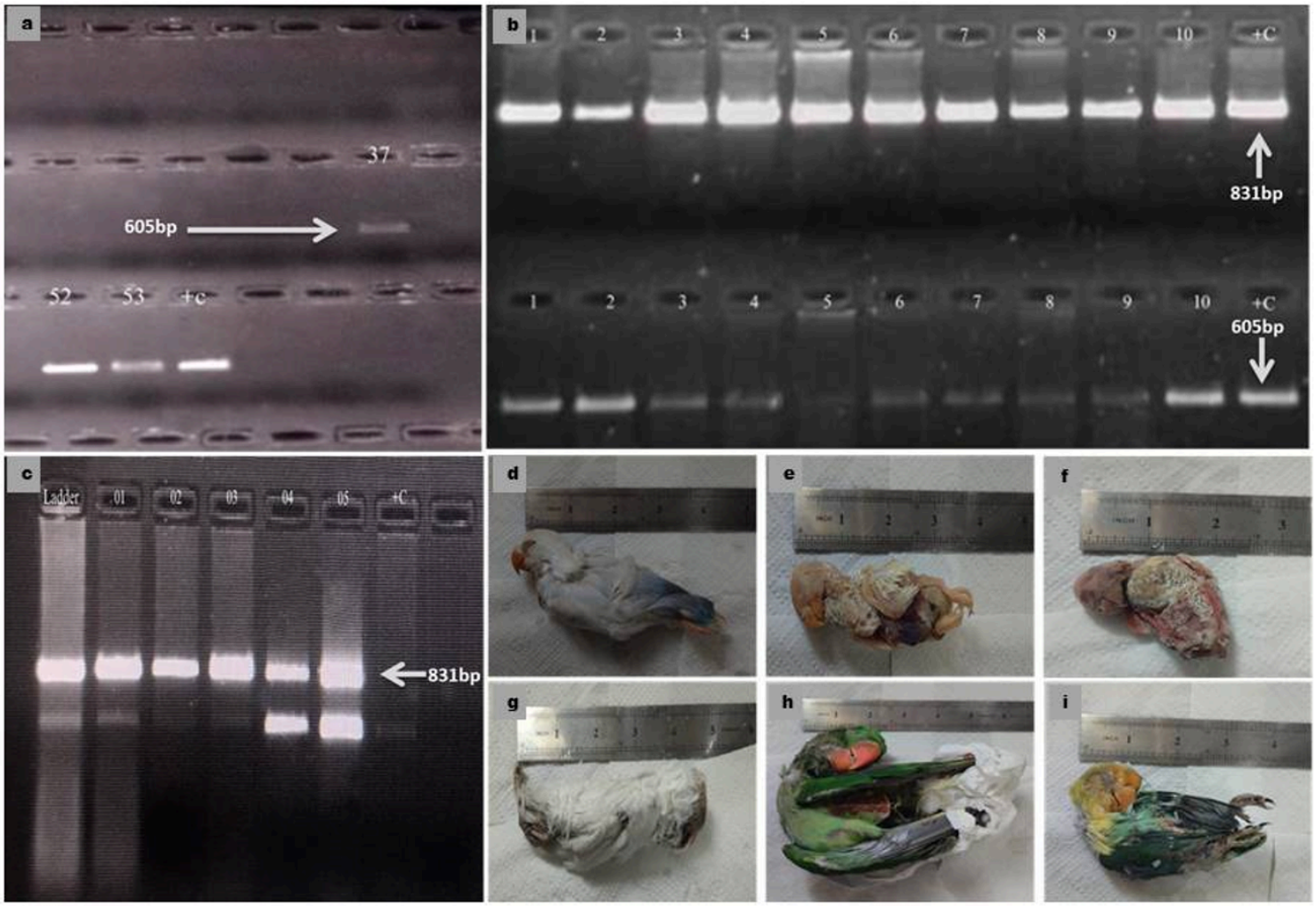


Figure 1

PCR-based detection of Avian Polyomavirus (APV) and Psittacine Beak and Feather Disease Virus (PBFDV) in psittacine birds and representative images of deceased birds examined for diagnostic testing

(a) PCR amplification of PBFDV showing a 605 bp band in lanes 37, 52, and 53

(b) PCR amplification of APV showing an 831 bp band in lanes 1–5, and co-infection cases showing both 831 bp (APV) and 605 bp (PBFDV) amplicons in lanes 1–10. +C indicates positive controls

(c) Additional APV-positive samples showing 831 bp amplification in lanes 01–05

(d–i) Representative psittacine birds submitted for diagnostic testing, including (d-g) lovebirds (Agapornis sp.), (i) a macaw parrot, and (h) a ring-neck parrot, exhibiting characteristic disease symptoms