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Investigation of molecular detection rate and associated risk factors of psittacine beak and feather disease virus in Psittaciformes in Iran

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Abstract: Psittacine beak and feather disease virus (PBFDV) is a significant viral pathogen affecting the development of beak and feather cells, leading to feather abnormalities in avian species. This study aimed to investigate the molecular detection rate of PBFDV among Psittaciformes in Iran and to evaluate the influence of factors such as sex, species, season, and bird origin on PBFDV prevalence. Feather samples were collected from 1335 Psittaciformes, including *Agapornis roseicollis*, *Pyrrhura molinae*, *Myiopsitta monachus*, *Aratinga solstitialis*, *Nymphicus hollandicus*, *Psittacula krameri*, *Psittacus erithacus*, *Poicephalus senegalus*, *Eos bornea*, and *Agapornis fischeri*, and subjected to polymerase chain reaction (PCR) for PBFDV detection. The overall PBFDV detection rate across all species was 56.2% (751/1335, 95% CI: 53.6%–58.9%). The results showed that *Agapornis roseicollis* had a 2.23-fold higher likelihood of PBFDV infection compared to the reference species, *Myiopsitta monachus* (95% CI: 1.36–3.66). Furthermore, a significant interaction effect between season and species was observed. During autumn, *Aratinga solstitialis* and *Pyrrhura molinae* were 10.00 (95% CI: 5.10–20.59) and 3.72 (95% CI: 2.28–6.08) times more likely to be infected with PBFDV compared to spring, respectively. These findings underscore a high prevalence of PBFDV in Iran's psittacine population. The study emphasizes the importance of such investigations and the exploration of various risk factors for disease control and prevention strategies. It also underscores the necessity of virological assessments prior to bird export and import.

Key words: Psittaciformes, psittacine beak and feather disease virus, risk factors, PCR

1. Introduction

Psittacine beak and feather disease virus (PBFDV) is the most commonly recognized viral causative agent of diseases affecting the skin and feathers of companion birds. It is responsible for clinical difficulties such as sudden death [1]. PBFDV belongs to the family Circoviridae, genus *Circovirus*. Its genome is an ambisense circular, single-stranded DNA approximately 2 kb in size. It contains two major open reading frames (ORFs) encoding the replication-associated protein (Rep) and the capsid protein (CP), respectively [2]. Psittacine beak and feather disease (PBFD) can manifest in acute and chronic forms. The acute form is characterized by a high mortality rate and severe clinical manifestations, primarily affecting young and newborn birds. The chronic form is more commonly reported and affects adult birds. Additionally, a subclinical infection form exists, where infected birds show no clinical signs yet can still shed the virus and infect other birds [3].

This viral agent poses challenges in treatment and can result in cross-transmission between bird species, leading to significant economic losses for breeders [4]. Since its initial detection in Australia five decades ago, outbreaks of PBFD in both wild populations and companion birds have been reported worldwide [5-7]. The virus spreads to birds through various routes, including direct contact, gastrointestinal or respiratory intake, and vertical transmission through eggs [8,9]. It infects a broad spectrum of birds, including Psittaciformes, Columbiformes, Passeriformes, and Anseriformes [10]. PBFDV primarily targets growing cells in beaks, claws, and feather follicles, resulting in feather malformation and loss. Additionally, it affects the bursa fabricii and the thymus, leading to immunosuppression due to decreased lymphocyte production [11]. One of the most significant challenges in controlling, preventing, and identifying the sources of the disease, alongside addressing reservoir and carrier birds, is the identification of infected birds. While PBFDV has been previously identified using serological methods, these traditional approaches cannot accurately determine the prevalence of the disease in different infected areas [7,12,13]. New molecular methods

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and genotyping techniques not only provide precise assessments of virus prevalence but also facilitate the identification of new genotypes [14-16]. In recent years, there has been a growing interest in keeping ornamental birds in Iran, leading to a notable increase in exchanges, trades, and breeding activities involving these birds within the country. Consequently, there has been a rise in clinical referrals with suspected symptoms of this disease to veterinary clinics in Iran [17]. Therefore, conducting a comprehensive study with a sufficient number of samples to ascertain the detection and distribution of PBFDV among ornamental bird populations appears imperative. Therefore, the present study was undertaken as an epidemiological investigation to evaluate the frequency of PBFDV in clinically healthy birds and assess its associated risk factors in Iran.

2. Materials and methods

2.1. Ethics approval

All experimental procurers were approved by the Ethics Committee of Amol University of Special Modern Technologies, Iran (IR.AUSMT.REC.1402.14).

2.2. Size, type of samples, and data collection

In this cross-sectional study, a total of 1335 samples were examined over the course of 1 year (from March 2021 to February 2022). The target population consisted of psittacine birds from 267 breeding and sale centers across 14 provinces of Iran, including green-cheeked parakeets (*Pyrrhura molinae*), rosy-faced lovebirds (*Agapornis roseicollis*), monk parakeets (*Myiopsitta monachus*), sun parakeets (*Aratinga solstitialis*), cockatiels (*Nymphicus hollandicus*), African grey parrots (*Psittacus erithacus*), rose-ringed parakeets (*Psittacula krameri*), Senegal parrots (*Poicephalus senegalus*), red lories (*Eos bornea*), and Fischer's lovebirds (*Agapornis fischeri*). To determine the required sample size for the study, considering a PBFDV prevalence of 18.18% based on a previous study in Iran, a confidence level of 95%, and a precision of 0.05, it was determined that a minimum of 229 ornamental bird breeding and sales centers should be sampled. Ultimately, samples were collected from 267 centers [17]. At each center, five birds were randomly selected from various cages, constituting an independent sample (a total of 1335 individual samples). Subsequently, five feathers were plucked from each selected bird and stored in separate sterile containers before being sent to the laboratory for molecular detection. To determine the sex of the captive birds, samples were sent to the reference veterinary laboratory. Sampling was conducted according to the protocol established by the present study, with coordination and cooperation from veterinarians in the aforementioned provinces. During sampling, veterinarians assessed the health status of the birds. Before sending the samples, a structured questionnaire was dispatched to breeding or sale centers to gather information on the age and species of the birds. This sample size was also deemed sufficient to investigate risk factors, providing more than 95% confidence in detecting a significant difference in the odds ratio of two for the factor of interest [18].

2.3. DNA extraction

DNA extraction from feather roots was performed using a DNA extraction kit (Sinaclon, Iran) following the manufacturer's instructions. In brief, 50 mg of each sample (feather root) was mixed with 20 µL proteinase K (Sinaclon, Iran) and incubated at 55 °C for 10 min. After centrifugation of the mixture at 13,000 rpm, the supernatant was combined with 200 µL binding solution in a new tube and incubated again at 60 °C for 10 min. Subsequently, 100 µL isopropanol was added to the tube, and the liquid was transferred into a binding column, followed by centrifugation at 8000 rpm for 1 min. This process was repeated using 500 µL for both washing buffers one and two. Finally, DNA was precipitated using 40 µL elution buffer and centrifuged at 13,000 rpm for 1 min. The extracted DNA and isolates were stored at –20 °C for future use in subsequent steps of the study.

2.4. Polymerase chain reaction (PCR)

A DNA fragment (717 bp) of the PBFDV genome was amplified using primers described by Ypelaar et al. (1999), with the forward sequence of 5`-AACCCTACAGACGGCGAG-3` and the reverse primer sequence of 5`-GTCACAGTCCTCCTTGTACC-3` [19]. PCR was performed in a final volume of 25 µL, comprising 12.5 µL of PCR master mix (Sinaclon, Iran), 1 µL (0.4 µM) of both forward and reverse primers, and 2 µL of DNA. All components were provided by Sinaclon Corporation, Iran. The PCR product was then assessed by electrophoresis in 1.5% agarose gel alongside a 100 bp DNA marker. To mitigate the risk of PCR product contamination, distinct areas were designated for the preparation of the PCR reaction buffer, viral DNA extraction, and PCR product analysis. Additionally, sterile filter tips were employed, work benches were decontaminated using UV light, and negative controls were included in every PCR run. Sinaclon, Iran was utilized to evaluate the obtained DNA amplicons. All PCR reactions were meticulously prepared in a designated and separate location within a laboratory hood, utilizing sterilized materials and instruments to prevent contamination. Sterile distilled water served as a negative control. To initiate the reaction, clinically positive samples were obtained from clinical centers and veterinary laboratories. Once the desired band was identified and the reaction set up, the initial positive sample was employed as a positive control for all subsequent reactions.

2.5. Statistical analysis

The statistical analysis was conducted using a binary

logistic regression model. In this model, age (categorized as under 1-year-old or greater than 1-year-old), sex, species, season of sampling (spring: April–June, summer: July–September, autumn: October–December, winter: January–March), and origin of the birds (from breeding or sales centers) were considered predictors, while the PCR test results served as the dependent variable. The detection rate of PBFDV in Psittaciformes and the corresponding 95% confidence interval were calculated. Univariable analysis for all independent variables was performed using the chi-square test.

Multivariable analysis was conducted using a logistic regression test. Variables with a p-value less than 0.2 in univariable analysis were selected to enter the multivariable analysis. However, the age variable was not included in the final model due to more than 10% missing data [20,21]. Potential interaction terms between independent variables were tested through two-way interactions in the final model. To summarize the model, backward elimination (likelihood ratio) was employed. Subsequently, the goodness-of-fit of the final logistic regression model was assessed using the Hosmer-Lemeshow test. Frequency distribution, odds ratios representing the strength of association, and the p-values of independent variables were calculated and estimated based on a multivariable logistic regression model. The analysis was performed using commercially available software (Stata version 16 statistical software, Stata Corp, College Station, TX, USA). A p-value under 0.05 was considered statistically significant. To ensure an adequate sample size for analysis, six bird species (*Nymphicus hollandicus*, *Psittacula krameria*, *Psittacus erithacus*, *Poicephalus senegalus*, *Eos bornea*, and *Agapornis fischeri*) were combined and labeled as "other species." Additionally, the statistical analysis focused on the four most common ornamental species in Iran: the green-cheeked parakeet, rosy-faced lovebird, monk parakeet, and sun parakeet.

3. Results

3.1. The detection rate of PBFDV based on PCR method

In the current study, a total of 1335 samples were tested, comprising *Agapornis roseicollis* (490), *Pyrrhura molinae* (457), *Myiopsitta monachus* (125), *Aratinga solstitialis* (75), *Nymphicus hollandicus* (55), *Agapornis fischeri* (52), *Psittacus Erithacus* (34), *Psittacula krameria* (19), *Poicephalus senegalus* (16), and *Eos bornea* (12). The overall detection rate of PBFDV was 56.2% (751/1335, 95% CI: 53.6–58.9%). Table 1 presents the positive rate of PBFDV in Psittaciformes across different provinces of Iran. The majority of samples (88%) were collected from Mazandaran, Golestan, Khuzestan, Yazd, and Tehran provinces. Notably, the highest positive rates of PBFDV were observed in Khorasan Razavi (81.2%, 95% CI: 62.1–100%), Qazvin (70%, 95% CI: 49.9–90.1%), Fars (67.7%, 95% CI: 51.3–84.2%), and Qom (66.6%, 95% CI: 40–93.3%) provinces, respectively (Figure).

Table 2 demonstrates the positive rate of PBFDV according to the studied independent variables The positive rate of PBFDV was different in various species and there was a significant relationship between bird species and the positive rate of PBFDV (p = 0.001). Specifically, the highest positive rates were observed in *Agapornis roseicollis* (65.7%, 95% CI: 61.5–69.9%), *Pyrrhura molinae* (50.5%, 95% CI: 46–55.1%), *Aratinga solstitialis* (45.3%, 95% CI: 34–56.6%), and *Myiopsitta monachus* (40%, 95% CI: 31.4–48.5%) species, respectively.

3.2. Factors associated with PBFDV based on univariable analysis

Univariable statistical analysis revealed that *Agapornis roseicollis* and *Pyrrhura molinae* had a higher risk of testing positive for PBFDV compared to *Myiopsitta monachus*, with odds ratios of 3.0 (95% CI: 1.92–4.30) and 1.53 (95% CI: 1.03–2.29), respectively.

Age data were available for only 236 birds, and an analysis was performed to examine the relationship between age and the positive rate of PBFDV in this subset. Younger birds aged under 1-year-old exhibited a lower positive rate of PBFDV compared to older birds, with rates of 52.3% (95% CI: 44.9–59.8%) versus 64.1% (95% CI: 52.3–75.8%). However, when accounting for odds ratios to understand the association between age and PBFDV infection, no statistically significant difference was observed.

In our study, males exhibited a higher positive rate for PBFDV at 57.8% (95% CI: 54.0–61.6%) compared to females at 54.7% (95% CI: 51.0–58.4%). However, when considering the odds ratio to assess the association between sex and PBFDV infection, no statistically significant difference was found.

There is a statistically significant relationship between the positive rate of PBFDV and the season of the year. The positive rate in autumn was higher than in other seasons, at 69.1% (95% CI: 64.2–74.0%), and this difference was statistically significant (p = 0.001). Univariable statistical analysis revealed that autumn and summer had a greater risk of testing positive for PBFDV compared to spring, with odds ratios of 2.51 (95% CI: 1.87–3.37) and 1.64 (95% CI: 1.21–2.22), respectively.

The positive rate of PBFDV in ornamental birds residing in sales centers (495) was higher compared to those in breeding centers (840), at 57.6% (95% CI: 53.1–62%) versus 42.4% (95% CI: 38.1–46.9%), respectively. However, there was no statistically significant difference between the two groups.

Table 1. Estimated detection rate of PBFDV among Psittaciformes in Iran based on PCR detection of BFDV from feather samples by province (from March, 2021 to February, 2022).

Province	Number of tested birds	Positive samples (%)	Negative samples (%)
Alborz	28	13 (46.4)	15 (53.6)
Bushehr	9	5 (55.5)	4 (44.4)
East Azerbaijan	12	4 (33.3)	8 (66.7)
Fars	31	21 (67.7)	10 (32.3)
Ghazvin	20	14 (70.0)	6 (30.0)
Ghom	12	8 (66.6)	4 (33.3)
Gilan	18	10 (55.5)	8 (44.4)
Golestan	128	70 (54.6)	58 (45.3)
Kerman	16	7 (43.7)	9 (56.3)
Khorasan Razavi	16	13 (81.2)	3 (18.8)
Khuzestan	82	38 (46.3)	44 (53.7)
Mazandaran	824	474 (57.5)	350 (42.5)
Tehran	69	35 (50.7)	34 (49.3)
Yazd	70	39 (55.7)	31 (44.3)
Total	**1335**	**751 (56.2)**	**585 (43.8)**

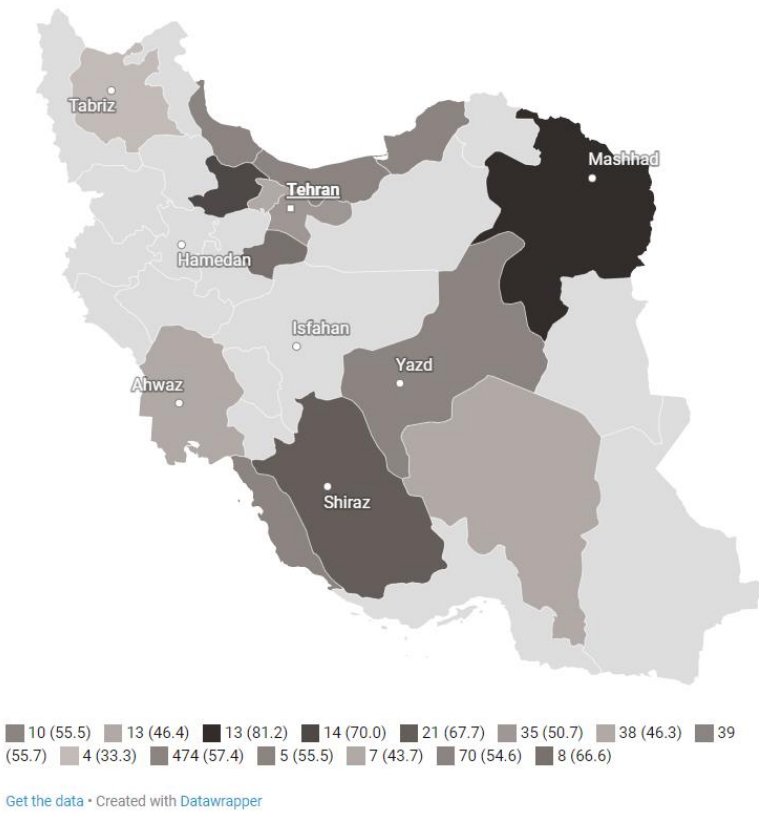

Figure. Distribution of PBFDV-positive birds across 14 provinces studied in Iran. The geographical density of PBFDV is depicted using a color intensity scale; darker colors indicate higher density. The image was generated by Datawrapper (https://app.datawrapper.de).

Table 2. Univariable analysis of some effective risk factors on the detection rate of PBFDV positivity among Psittaciformes in Iran.

Variable	Category	Number of tested birds	Positive (%)	[b]OR (95% CI)	[c]p-value
Species	*Myiopsitta monachus*[a]	125	50 (40.0)	1	-
	Aratinga solstitialis	75	34 (45.3)	1.24 (0.69–2.21)	0.46
	Pyrrhura molinae	457	231 (50.5)	1.53 (1.03–2.29)	0.03
	Agapornis roseicollis	490	322 (65.7)	3.0 (1.92–4.30)	0.001
	Other species[d]	188	113 (60.1)	2.30 (1.43–3.61)	0.001
Age (years)	≤1[a]	172	90 (52.3)	1	-
	>1	64	41 (64.1)	1.62 (0.90–2.93)	0.10
Sex	Female[a]	680	372 (54.7)	1	-
	Male	655	379 (57.8)	1.13 (0.91–1.40)	0.25
Season	Spring[a]	459	216 (47.1)	1	-
	Summer	272	162 (59.5)	1.64 (1.21–2.22)	0.001
	Autumn	340	235 (69.1)	2.51 (1.87–3.37)	0.001
	Winter	264	138 (52.3)	1.23 (0.91–1.67)	0.17
Origin of the birds	Breeding centers[a]	840	466 (55.5)	1	-
	Sales centers	495	285 (57.6)	1.08 (0.86–1.35)	0.48

[a]Reference group. [b]Odds ratio (confidence interval for OR). The odds ratio of each group is compared to the reference group. [c]$p < 0.05$ was considered statistically significant. [d]Other species (*Nymphicus hollandicus*, *Psittacula krameria*, *Psittacus erithacus*, *Poicephalus senegalus*, *Eos bornea*, and *Agapornis fischeri).*

In the univariable analysis, three factors were entered into the multivariable model: season, species, and the interaction term between season and species.

3.3.Factors associated with PBFDV based on multivariable analysis

Table 3 presents the results of the multivariable analysis. Regarding the relationship between the independent variables affecting the positive rate of PBFDV in ornamental birds, the *Agapornis roseicollis* species exhibited a statistically significant association. The odds ratio for this species was 2.23 times higher (95% CI: 1.36–3.66) compared to the reference group (*Myiopsitta monachus*) (p = 0.001). Moreover, the interaction effect of season × species in the multivariable analysis was found to be significant in the final model. Upon estimation of odds ratios for variables involved in interaction terms, it was evident that the interpretation differed between the model with interaction terms and the model without interaction terms. Consequently, our results revealed that during the autumn season, *Aratinga solstitialis* and *Pyrrhura molinae* species were 10.00 (95% CI: 5.10–20.59) and 3.72 (95% CI: 2.28–6.08) times more likely to be infected with PBFDV

Table 3. Multivariable logistic regression analysis of some effective risk factors on the detection rate of PBFDV among Psittaciformes in Iran.

Variable	Category	Number of tested birds	Beta coefficient	[c]OR (95% CI)
Species	*Myiopsitta monachus*[a]	125	-	1
	Aratinga solstitialis	75	0.49	1.05 (0.48–2.27)
	Pyrrhura molinae	457	–0.06	0.93 (0.58–1.50)
	Agapornis roseicollis	490	0.80	**2.23** (1.36–3.66)
	Other species[d]	188	0.58	1.80 (0.94–3.41)
Season × Species[b]	Summer × *Aratinga solstitialis*	8	–1.58	0.20 (0.02–1.84)
	Summer × *Pyrrhura molinae*	93	0.88	2.42 (0.92–4.05)
	Summer × *Agapornis roseicollis*	106	0.10	1.10 (0.65–1.86)
	Summer × Other species	34	0.99	2.70 (0.99–7.03)
	Autumn × *Aratinga solstitialis*	8	2.30	**10.00** (5.10–20.59)
	Autumn × *Pyrrhura molinae*	123	1.31	**3.72** (2.28–6.08)
	Autumn × *Agapornis roseicollis*	137	0.41	1.51 (0.92–2.50)
	Autumn × Other species	49	0.94	2.57 (0.88–5.95)
	Winter × *Aratinga solstitialis*	25	0.27	1.31 (0.46–3.73)
	Winter × *Pyrrhura molinae*	64	–0.24	0.78 (0.42–1.43)
	Winter × *Agapornis roseicollis*	110	0.53	1.70 (0.99–2.92)
	Winter × Other species	51	–0.54	0.58 (0.27–1.26)
Constant	-	-	–0.40	0.66

[a]Reference group.
[b]*Myiopsitta monachus* species and spring is considered the reference group.
[c]Odds ratio (confidence interval for OR). A confidence interval is meaningful when it does not include the number one and the odds ratio of each group is compared to the reference group.
[d]Other species (*Nymphicus hollandicus*, *Psittacula krameria*, *Psittacus erithacus*, *Poicephalus senegalus*, *Eos bornea*, and *Agapornis fischeri)* Hosmer-Lemeshow χ^2 (8) = 0.05, p-value = 0.91.

compared to other seasons, respectively ($p < 0.05$). The Hosmer and Lemeshow goodness-of-fit test for the final model indicated that the fit of the binomial logistic regression model was satisfactory ($p = 0.91$).

4. Discussion

PBFD is a highly contagious and potentially fatal disease in birds, with no known cure or vaccine. Its widespread prevalence is observed alongside the expanding breeding and maintenance of ornamental birds across various countries worldwide. However, it seems that the main source of this infection is still wild psittacine and nonpsittacine birds [5,13]. Previous reports indicate that subclinical and asymptomatic infected birds can serve as reservoirs for the disease, facilitating the spread of the virus through feathers, dust, and fecal matter [22].

This investigation focused on clinically healthy birds to investigate the distribution and presence of asymptomatic infection. Our study revealed a relatively high prevalence of PBFDV positivity among tested psittacine birds in Iran (56.2%). Comparable studies in other regions have shown conflicting results. Notably, all investigators employed similar sampling and diagnostic procedures. Our findings exceeded those reported in Türkiye (48.7%), UAE (45.13%), Germany (39.2%), Taiwan (41.2%), Czech Republic (21.5%), and Italy (8.05%) [5-6,11,23-25]. These disparities suggest that the prevalence of PBFDV in ornamental birds can vary significantly across regions and populations, potentially influenced by factors such as management practices and environmental conditions.

Another intriguing aspect of this study is the detection of PBFDV-positive birds in all surveyed provinces. Previous studies by Haddadmarandi et al. (2018) and Dolatyabi et al. (2022) indicated its presence in the majority of provinces in Iran, and our findings further underscore the widespread distribution of PBFDV across the country [17,26]. This observation may be attributed to the growing trend of breeding and pet ownership of these birds, as well as the trafficking of birds by vendors throughout the country, which potentially contributes to the increased spread of PBFDV nationwide.

In the next step, we analyzed the positive rate of PBFDV across various species. As indicated in Table 1, *Agapornis roseicollis* (65.7%) followed by *Pyrrhura molinae* (50.5%) exhibited the highest positive rates and were at higher risk of testing positive for PBFDV compared to other examined species. In another independent study conducted by Dolatyabi et al. (2022), the majority of PBFDV-positive cases were found within the *Agapornis* and *Nymphicus* genera. Additionally, other genera such as *Melopsittacus*, *Pyrrhura*, and *Psittacus* also demonstrated a considerable percentage of positive cases [26]. Furthermore, Monrinha et al. (2020) conducted a study analyzing the prevalence of PBFDV in populations of *Psittacula krameri* (rose-ringed parakeets) and *Myiopsitta monachus* (monk parakeets) in Southern Spain. They found that approximately 33% of rose-ringed parakeets and 37% of monk parakeets sampled tested positive for PBFDV, despite neither species exhibiting any disease symptoms [27]. The abundance of observations and populations of certain species in Iran may contribute to the higher probability of PBFDV-positive cases in these species compared to others. Indeed, previous studies have suggested the influence of bird species on the prevalence rate of PBFDV [28-30]. However, it is noteworthy that in the present study, the prevalence of PBFDV among nonparrot populations was not assessed. Moreover, the virus demonstrates flexible host switching and recombination, implying that all susceptible hosts, both domestic and wild, may be affected by closely or distantly related reservoir species [31]. Additionally, there are numerous reports of PBFDV infection in nonpsittacine birds. Recent research has also indicated a relatively high prevalence of this disease among nonpsittacine birds in Australia (38.1%), New Zealand, and other countries [13,31-32]. These findings raise concerns about the transmission of the virus from psittacine birds to wild nonpsittacine birds following the escape or release of carrier birds into the wild.

Previous studies have suggested seasonal fluctuations in PBFD infections among certain bird species [28,33]. Consistent with these findings, the results of our study indicate a significant likelihood of PBFD infection being observed in the autumn season, with *Aratinga solstitialis* and *Pyrrhura molinae* birds being 10 and 3.72 times more likely to be infected during this season compared to others. Among the total studied birds, 656 (49.1%) were males and 680 (50.9%) were females. Although the positive rate of infection was higher in male birds (57.8%), our analysis did not detect a significant role of bird sex in the frequency of infection (Table 2). Additionally, our observations revealed relatively high bird density in cages and inadequate air conditioning in sales and breeding centers, respectively. Therefore, further investigations are imperative to enhance our understanding of the transmission dynamics, risk factors, and effective treatments for PBFDV in birds. Implementation of disease control and prevention strategies is crucial, given the widespread occurrence of this virus in the psittacine population throughout Iran.

The present study has several limitations primarily related to selection bias. Additionally, focusing on the four most prevalent ornamental species in Iran may limit the generalizability of the findings to the broader Psittaciformes population, including nonornamental or wild Psittaciformes, potentially compromising the sample's representativeness. Another limitation is that the methodology's sensitivity and specificity may have led to

an underestimation of PBFDV prevalence due to potential false negatives. While a longitudinal study design is recommended for improved causal inference, our model offers valuable insights, and the established relationships can serve as a basis for future longitudinal studies. Moreover, the lack of comprehensive clinical data hinders a thorough understanding of the clinical implications of PBFDV, emphasizing the need for longitudinal investigations to enhance causal inference. Therefore, future studies should take these limitations into account. Furthermore, future research endeavors could incorporate phylogenetic analysis to explore the genetic diversity of PBFDV strains and potential transmission patterns.

5. Conclusion

The overall detection rate of PBFDV highlights its widespread distribution among psittacine birds. Given that psittacine birds are not native to Iran, further investigations are warranted to assess the potential role of bird importation from native source countries. Moreover, it is crucial to raise awareness among bird owners, breeders, and traders regarding the risk factors associated with PBFDV. Promoting appropriate preventive measures can significantly contribute to reducing the incidence and prevalence of the disease. In conclusion, our study did not find any association between age and sex positivity for PBFDV across all bird species tested. However, our analyses indicate that the autumn season represents an important risk factor for PBFDV infection. Furthermore, the risk of infection with this virus is higher in the *Agapornis roseicollis* species compared to other species. Additionally, PBFDV has the potential to infect and cause disease in nonpsittacine birds. Therefore, conducting further studies to elucidate the occurrence of PBFDV in nonpsittacine populations is essential.

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Conflict of interest

The authors declare no conflicts of interest.

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