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Original article

MOLECULAR CHARACTERISATION OF POLYOMAVIRUS IN PARROTS IN IRAN

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Summary

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Avian polyomavirus (APV) is a highly significant viral threat to caged birds. The disease results in substantial financial losses for farmers and pet store owners annually. Identifying the cause of the disease is crucial, as infected parrots may display similar symptoms to those affected by other viral diseases like psittacine beak and feather disease. The aim of this study was the molecular detection and characterisation of the avian polyomavirus in parrots of Iran and to explore the correlation between the infection and the bird species. In addition, a two-step nested-PCR technique was developed and implemented in place of traditional single-step methods to enhance the sensitivity of virus detection. A total of 85 cloacal samples were collected from four different species of parrots. For amplification, two sets of primers to target a specific region of the VP1 gene were used in a two-step nested PCR protocol. The PCR products were sequenced, and phylogenetic analysis was conducted to investigate the source of the virus across studied bird species. The obtained results demonstrated that 11.76% of the sampled and tested avian population were positive based on the PCR and gel electrophoresis. Direct sequencing of the samples showed that 0.98% of amplified nucleotides had mutation in one or more samples. Finally, the phylogenetic analysis revealed that different strains of APV were circulating in rosy-face lovebirds and budgerigars in Iran. In conclusion, our findings confirmed the presence of APV in parrot flocks in Iran. Additionally, the APV *VP1* gene demonstrated its potential as a valuable target for future identification and molecular analyses of the APV.

Key words: avian polyomavirus, nested-PCR, phylogenetic analysis, sequencing, *VP1* gene

INTRODUCTION

Polyomavirus is a non-enveloped double-stranded DNA virus and contains a circular genome of about 5,000 base pairs. Its genetic material is packed inside a 40–50 nm viral capsid, which has a polyhedral shape. The capsid is made up of 72 protein structures called pentameric capsomers. These capsomers, formed by a protein called VP1, can self-assemble into a closed polyhedron. Additionally, each

VP1 pentamer is associated with either VP2 or VP3, which are two other proteins that make up the capsid (Moens *et al.*, 2017). A typical polyomavirus carries a genome that encodes 5 to 9 proteins. These proteins are categorised into two transcription regions known as the early and late regions. When the virus infects a host, these regions are transcribed to facilitate the viral infection process (Saribas *et al.*, 2019). The life cycle of polyomavirus begins by entering the host cell. The receptors on the surface of the host cells that the polyomavirus interacts with are typically sialic acid residues found in glycans, commonly known as gangliosides. To attach to the host cells, the VP1 protein of the polyomavirus binds to the sialylated glycan present on the cell surface. In certain strains of the virus, there may also be additional interactions with other components on the cell surface (Ströh *et al.*, 2020).

Avian polyomavirus, commonly known as APV, affects various species of birds. It belongs to the *Polyomaviridae* family and primarily targets psittacine birds, including parrots, cockatoos, and macaws. APV is a highly contagious and potentially fatal virus that can have devastating effects on avian populations (Lafferty *et al.*, 1999). First discovered in the 1970s, APV gained attention due to its rapid spread and high mortality rate among affected birds. It is transmitted through direct contact with infected birds, contaminated surfaces, or even through the air (Adiguzel *et al.*, 2020). This makes it a significant concern for both pet bird owners and aviculturists who work with these species. One of the most challenging aspects of APV is its ability to remain dormant in infected birds, making it difficult to detect until symptoms appear (Phalen, 1998). Infected birds may carry the virus without exhibiting any signs, serving as carriers and potential sources of transmission. This makes it crucial to implement strict biosecurity protocols to prevent the spread of the virus within aviaries, bird sanctuaries, and breeding facilities (Lafferty *et al.*, 1999; Johne & Müller, 2007). Many birds may be infected with the avian polyomavirus, but only a certain and sometimes small percentage of them develop the disease. The symptoms of APV can vary depending on the affected bird species, age, and general health. However, common signs include feather abnormalities, weight loss, dehydration, regurgitation, diarrhoea, and lethargy. Some birds may also develop neurological symptoms such as tremors, loss of balance, and difficulty in coordination. These symptoms can progress rapidly, leading to severe illness and, in some cases, death (Ma *et al.*, 2019; Adiguzel *et al.*, 2020).

Diagnosing APV involves a combination of physical examination, medical history evaluation, and laboratory tests. Veterinarians often perform blood tests, DNA analysis, or viral culture to confirm the presence of the virus. Early detection is crucial for implementing appropriate treatment and preventive measures to control the spread of the infection (Latimer *et al.*, 1993). It is crucial to highlight that APV not only affects pet birds but also poses a threat to wild bird populations. The virus can be transmitted to wild birds through contact with infected captive birds, making it essential for aviculturists to take responsibility for preventing the spread of the disease beyond their own facilities (Enders *et al.*, 1997).

Currently, there is no specific antiviral treatment available for APV. The focus of treatment is mainly supportive care, aiming to alleviate symptoms and strengthen the bird's immune system. This may in-

clude fluid therapy, nutritional support, and medication to manage secondary infections. However, the effectiveness of treatment may vary depending on the stage of the disease and the overall health of the affected bird. Prevention is key when it comes to managing APV (Topor, 1999). Bird owners and aviculturists should prioritise implementing strict biosecurity measures to minimise the risk of infection. This includes regular disinfection of cages and aviaries, quarantine protocols for new birds, and careful screening of potential carriers. Vaccination for APV is also available in some regions and considered as part of a comprehensive preventive strategy (Ritchie *et al.*, 1998).

It is crucial to determine the cause of the disease, as parrots may exhibit similar symptoms when infected with other viral diseases, such as psittacine beak and feather disease (PBFD). Also, there is no published study regarding the incidence of avian polyomavirus in parrot flocks of Iran. Therefore, the primary objective of this study was to identify the presence of the polyoma virus in parrots, and to explore the correlation between the virus's presence, and the bird species. Additionally, a two-step nested-PCR technique was developed and implemented in place of traditional single-step methods to enhance the sensitivity of virus detection.

MATERIALS AND METHODS

Sampling

A total of 85 cloacal samples from suspicious birds with general non-specific clinical signs such as feather abnormality, diarrhoea, and dehydration were taken. The age of all of the birds was between 3 days and 2 months including 23 cockatiels (*Nymphicus hollandicus*), 28 budgerigars (*Melopsittacus undulatus*), 25 rosy-faced lovebirds (*Agapornis roseicollis*), and 9 green-cheeked parakeets (*Pyrrhura molinae*). The mentioned samples were collected from different cities in Iran – Tehran, Rasht, Kashan, Isfahan, and Tabriz. The samples were labelled with numbers and the relevant information was recorded in an Excel file for further analysis.

DNA extraction

DNA extraction was performed using a total DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions. Briefly, 200 µL of each sample was transferred to a labelled 1.5 mL microtube. DNA extraction buffer (800 µL) was added to each microtube containing the obtained samples. Then, the microtubes were vortexed for 10 s. In the next step, the microtubes were placed in a dry bath for 45 min, the temperature of which was previously set at 65 °C.

Next, 300 µL of chloroform was added to each microtube. After adding chloroform to the microtubes and closing the microtube lid, they were turned upside down by movements in the form of an infinity sign so that the chloroform and the contents inside the microtube were slowly mixed together, and this was repeated several times. Then, the microtubes were centrifuged for 15 min at 13,000 rounds per minute (rpm) and temperature of 4 °C. Three separated phases, including a transparent and aqueous phase (containing DNA), an intermediate phase or interphase (containing protein), and an organic phase (containing lipid) were formed after the centrifugation. The supernatant was transferred to a new tube and isopropanol (1 mL) was added. The microtubes were transferred to the centrifuge and the previous settings (temperature 4 °C, 13,000 rpm for 15 min) were given to the ma-

chine. At the end of the work, small sediment pellets of the extracted DNA were observed at the bottom of the microtubes. The microtubes were placed upside down on a clean paper towel, and the remaining alcohol was allowed to dry. After a few minutes, 50 µL of DEPC water was added to the sediments and placed in a dry bath for 30 min to dissolve the sediment in the water and prepare the DNA for further tests and investigations. Using a spinner, the microtubes were spun so that all the contents concentrated at the bottom of the microtube.

At the end, to ensure the correct and sufficient concentration of the extracted DNA and the absence of any contamination, the samples were examined by a Nanodrop spectrophotometer (OneC Microvolume, Thermo Scientific, USA). The concentration of the samples was checked and the average OD of 260/280 and 260/230 was checked. Finally, extracted DNA was stored and frozen until the further analysis.

Primer designing

Table 1 shows the primer sequences (and their product size) that were used to amplify the partial *VP1* gene in APV. A pair of specific primers (APV1 F & APV1 R) were selected from a study published by Altan *et al.* (2016) for the amplification of the partial VP1. In the next step, Oligo7 software (https://www.oligo.net/) was used to design a pair of suitable forward and reverse primers for performing a two-step nested polymerase chain reaction (PCR). To do this, *VP1* gene was searched in the NCBI database (https://ncbi.nlm.nih.gov/) and the FASTA format of the available DNA sequences were transferred to the Oligo 7 software. Specific primers (APV2 F & APV2 R) were designed manually. After ensuring the appropriateness of the designed forward and reverse primers in terms of duplex formation, hairpin formation and mixed oligos, the sequences were transferred to the NCBI database for BLAST and the specificity of the designed primers was confirmed.

PCR procedures

A commercial PCR master mix (Sinaclon Co., Tehran, Iran) was used for performing the PCR. All reactions were carried out in a final volume of 25 µL. For the first pair of primers (first amplification step), the PCR mixture was pre-denatured at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 40 s. Also, 72 °C for 5 min was used as the final extension. A similar temperature programme was used for the second primer pair (nested-PCR step), except for the annealing step that used 60 °C as annealing temperature.

Table 1. Primer sequences (and their PCR product size) for the detection of APV that have been designed and utilised in this study

Primer name	Sequence	PCR product size (bp)	Reference
APV1 F	CAGCACAGAGGTACCGTGTT	831	Altan *et al.* (2016)
APV1 R	ATCAGAGCCCTGCATGCTTT		
APV2 F	CCTAATTGGGCCTGGGGAAG	339	Designed in this study
APV2 R	AAATAGCGTGGTAGGCCTCG		

Gel electrophoresis

PCR products were electrophoresed on a 1% agarose gel containing 10 µL of SYBR Green stain (70 V, 40 min). The formed bands were then examined by an electrophoresis device (Syngene Bio Imaging, UK).

Direct sequencing

The direct sequencing method was used to analyse positive PCR products. A total of 9 PCR products of positive specimens were purified using a purification kit (Bioneer, Cat. No. K-3034) and each sample was sequenced separately using specific forward and reverse primers. Sequencing was performed by Macrogen Inc. (Seoul, Korea) on an automated DNA sequencer, ABI 3730 XL. The sequences were then analysed by BLAST at the NCBI database and the levels of similarity and percentage of coverage among the sequences were investigated. Amino acid sequences were deduced from nucleotide sequences using Bioedit software package version 7.0.5.3.

Phylogenetic analysis

The sequences of the reference strains of avian polyomavirus with the following accession numbers: MH881333.1, AB453162.1, AB453161.1, KT203768.1, KT203767.1, KT203765.1, MT981254.1, M20775.1, AB453166.1, AY589492.1, AB453159.1, MK516256.1, AB453160.1, MG148345.1, MH881330.1, MH881331.1, MK061528.1, MN720915.1, MH881328.1, GU452537.1, and MH881327.1 were retrieved from the NCBI database. A part of the *VP1* gene belonging to the mentioned strains and Iranian strains (samples of this article) were used for the construction of a phylogenetic tree. Phylogenetic analysis was performed using MEGA 7 software (http://www.megasoftware.net) and a phylogenetic tree was constructed by the neighbour-joining method. Bootstrapping over 1000 replicates was done to assess the confidence level of the branch pattern.

RESULTS

PCR and gel electrophoresis

The results of the gel electrophoresis for the products of the first stage (APV1) and the second stage (APV2) PCR are shown in Fig. 1. The products of the first stage and the second stage formed clear bands with a molecular weight of 831 bp and 339 bp, respectively.

Table 2 shows the distribution of positive and negative samples based on the city and species of birds. Rosy-faced

Table 2. Positive and negative samples of polyomavirus in the studied parrots based on bird species and city

Species / City	Cockatiel		Budgerigar		Rosy-faced love bird		Green-cheeked parakeets	
	+	–	+	–	+	–	+	–
Tabriz	0	5	0	4	0	4	0	0
Kashan	1	3	2	5	0	5	0	3
Shiraz	0	1	0	4	0	5	0	2
Tehran	0	10	1	5	5	4	1	1
Rasht	0	3	0	7	0	2	0	2
Total	1	22	3	25	5	20	1	8

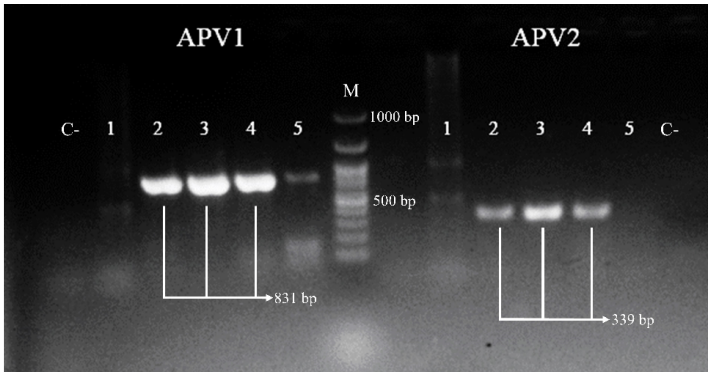

Fig. 1. Gel electrophoresis of the PCR products. The obtained samples are represented by numbers 1 to 5, the marker is represented by M, and the negative controls for each primer pair are represented by C-.

lovebirds had the highest incidence of positive samples with 5 positive samples, while the number of positive cases for cockatiels, budgerigars, and green-cheeked parakeets were 1, 3, and 1, respectively. Regarding the cities, Tehran had the highest incidence of positive cases (7 positive cases). Also, 3 positive samples were obtained from Kashan. The samples collected from Tabriz, Shiraz, and Rasht were free of APV.

In total, the incidence of contamination in cockatiels, budgerigars, rosy-faced lovebirds, and green-cheeked parakeets was 4.34%, 10.71%, 20%, and 11.1%, respectively. Out of the 85 samples examined in this study, 10 were positive. In general, 11.76% of collected samples from birds showing signs of the disease were diagnosed positive.

Sequencing and phylogenetic analysis

After alignment, a comparison between the reference sequence of MN720915 and sequenced samples of this study was performed. In general, four strains of polyomavirus were identified in parrots of Iran (Fig. 2).

Samples 15, 16, 18, 37 and 39 had a similar sequence. Compared to the reference sequence, these samples had multiple mutations in nucleotides 287, 329, 413, 518 and 599. Sample 12 was different from all other sequences and in addition to the mentioned mutations, also had a mutation in nucleotide number 84. Samples 13 and 17 had only one mutation in nucleotide number 56 compared to the reference sequence. Also, sample 14 had the same sequence as the reference sequence.

In total, 7 variable nucleotide regions were determined in the samples of this study. Considering the total number of 710 nucleotides, the percentage of mutations was calculated as 0.98%.

Based on the results of the phylogenetic analysis, the constructed tree included three main clusters that were separated from the ancestral node (Fig. 3). Iranian strains were placed in two separate clusters. Samples 13, 17, and 14 were in the same cluster. The rest of the samples were clustered together with the avian polyomavirus isolated from budgerigars in China. This analysis demonstrated that different strains of the avian polyomavirus are circulating in rosy-faced lovebirds and budgerigars of Iran.

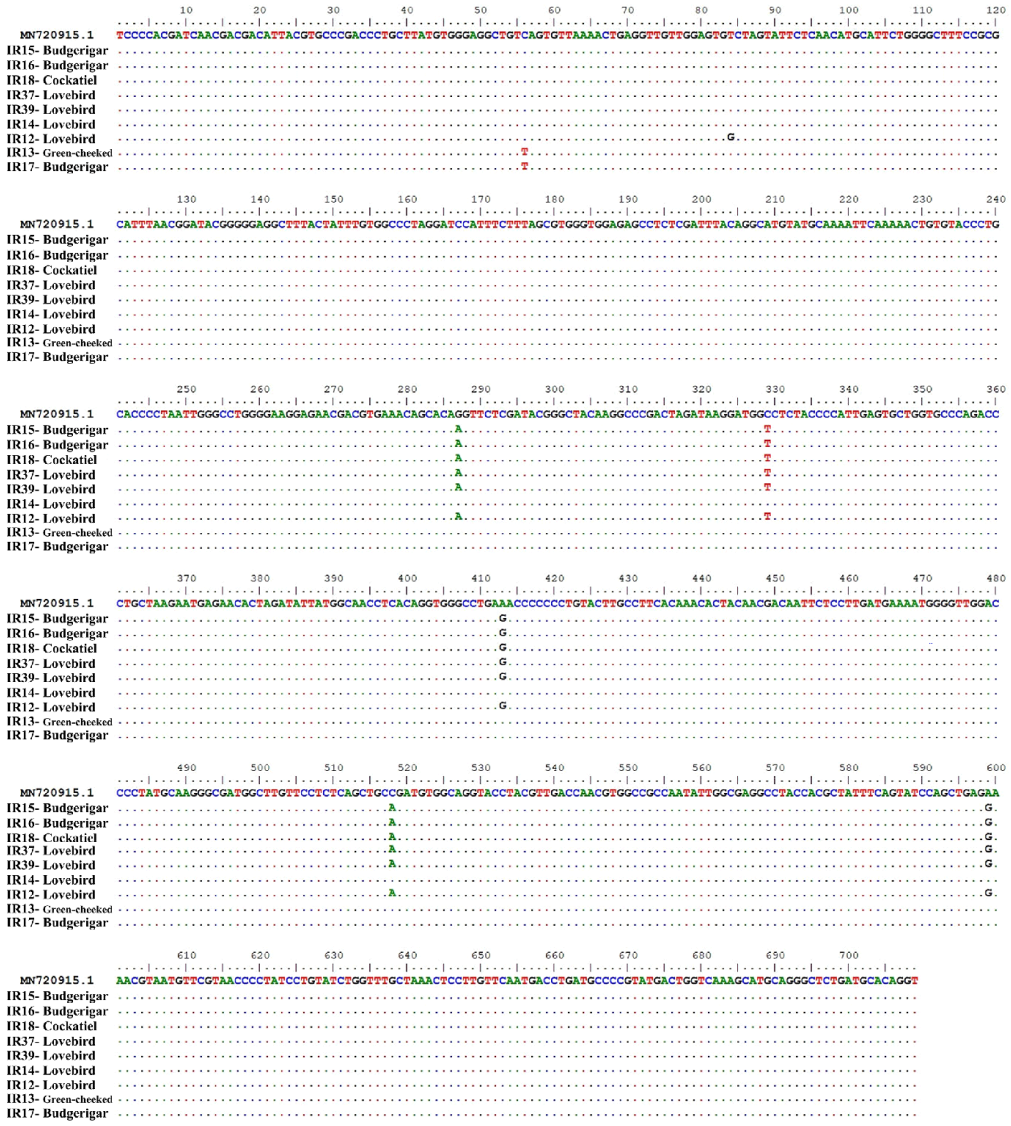

Fig. 2. Aligned sequences of the partial VP1 gene of avian polyomavirus that were amplified using the utilised specific primers. The sequences of local variants of Iran are shown aligned with the reference sequence of MN720915.1, obtained from GenBank.

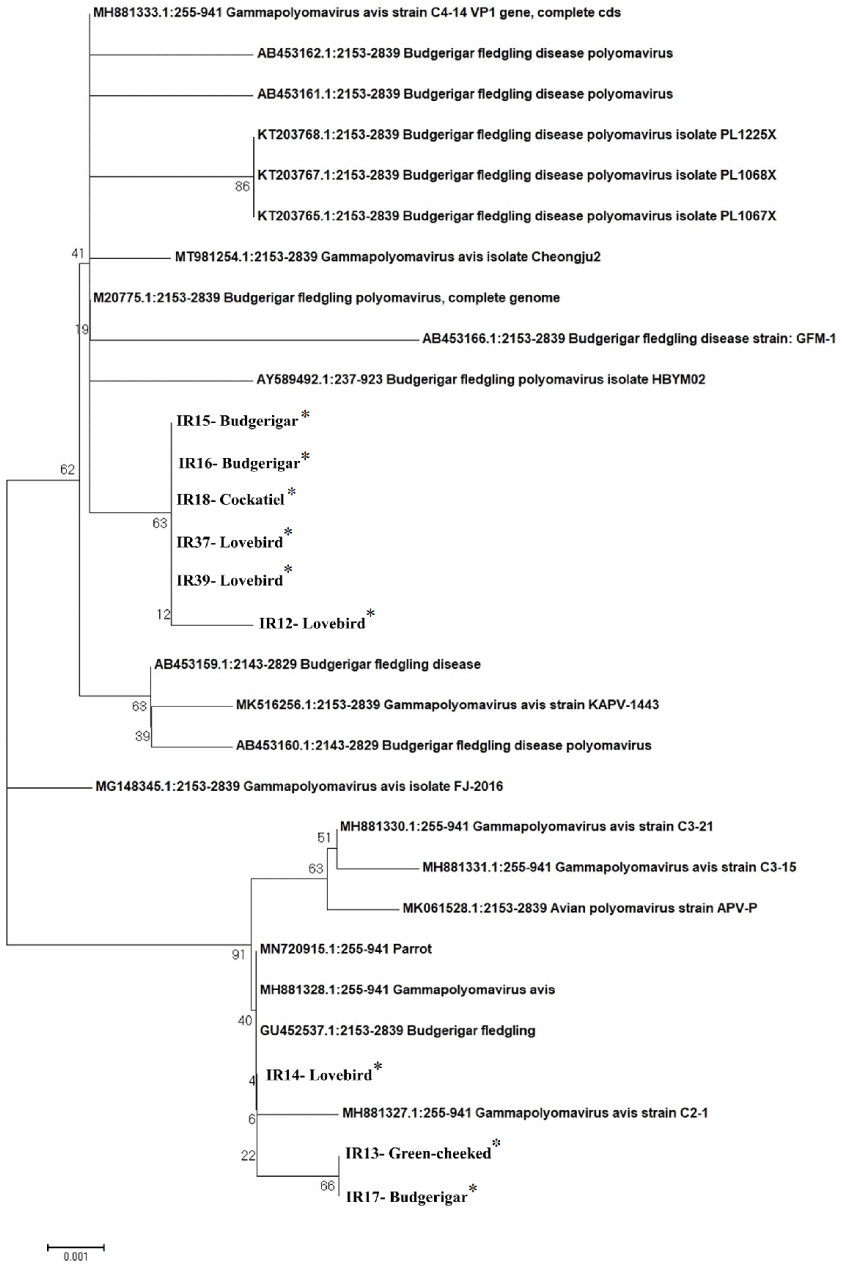

Fig. 3. Phylogenetic tree based on nucleotide sequence of the VP1 gene. Reference sequences of APV were used for phylogenetic tree construction. The evolutionary history was inferred using the neighbour-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The samples analysed in this study are indicated with an asterisk (*) symbol.

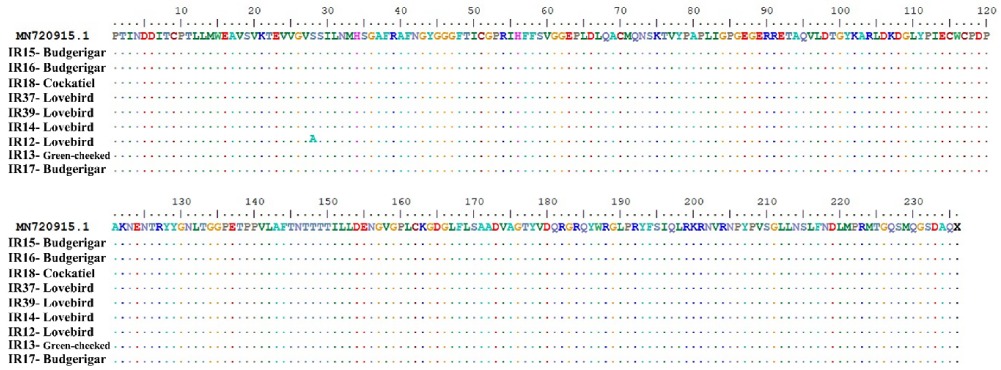

Fig. 4. Aligned amino acid sequences of avian polyomavirus partial VP1 gene. The sequences of local variants of Iran are shown aligned with the reference sequence of MN720915.1

In the following step, the deduced amino acid sequences of the samples were aligned again and the occurrence of mutation was checked. Based on the results, all the mutations occurred in the nucleotide sequences, except for one case, were of the silent type and have not led to the changes in the amino acid sequence of the *VP1* gene (Fig. 4). Only in sample number 12 (rosy-face lovebird) in position 28, serine has changed to alanine. Next, phylogenetic analysis was performed again based on the deduced amino acid sequences. Based on the results, all of the samples of this research were clustered together with the gamma polyomavirus strains (Fig. 5).

DISCUSSION

Polyomavirus is a highly infectious virus in parrots, and its prevalence is very high in several countries. The clinical symptoms of APV in young parrots are variable and mostly depend on the species, viral load, and age. Death without any predisease symptoms have been reported in young ones (Altan *et al.*, 2016). In a study, Katoh *et al.* (2010) investigated polyomavirus in different species of parrots and stated that large basophilic nuclear bodies were found in the spleen, liver and kidneys in the histopathological examinations of the organs of the affected parrots. In lovebirds, APV infection is often subclinical and birds die without showing signs and symptoms.

Polymerase chain reaction has become an extremely important tool for the diagnosis and control of infectious diseases. By using this method, a very small amount of virus can be amplified to a detectable level. When performed accurately, PCR can even identify as few as 100 virus copy numbers (Arnaout *et al.*, 2021). Apart from cloacal swabs, detection of APV DNA is also possible in the blood of birds that have been newly infected with APV. While some studies have published contradictory findings regarding the validity of blood-based PCR testing for APV, positive cases from both tests are considered reliable (Reavill & Dorrestein, 2018). In fact, consistent positivity in blood PCR tests has been observed in all cockatoos and conures that were intermittently posi-

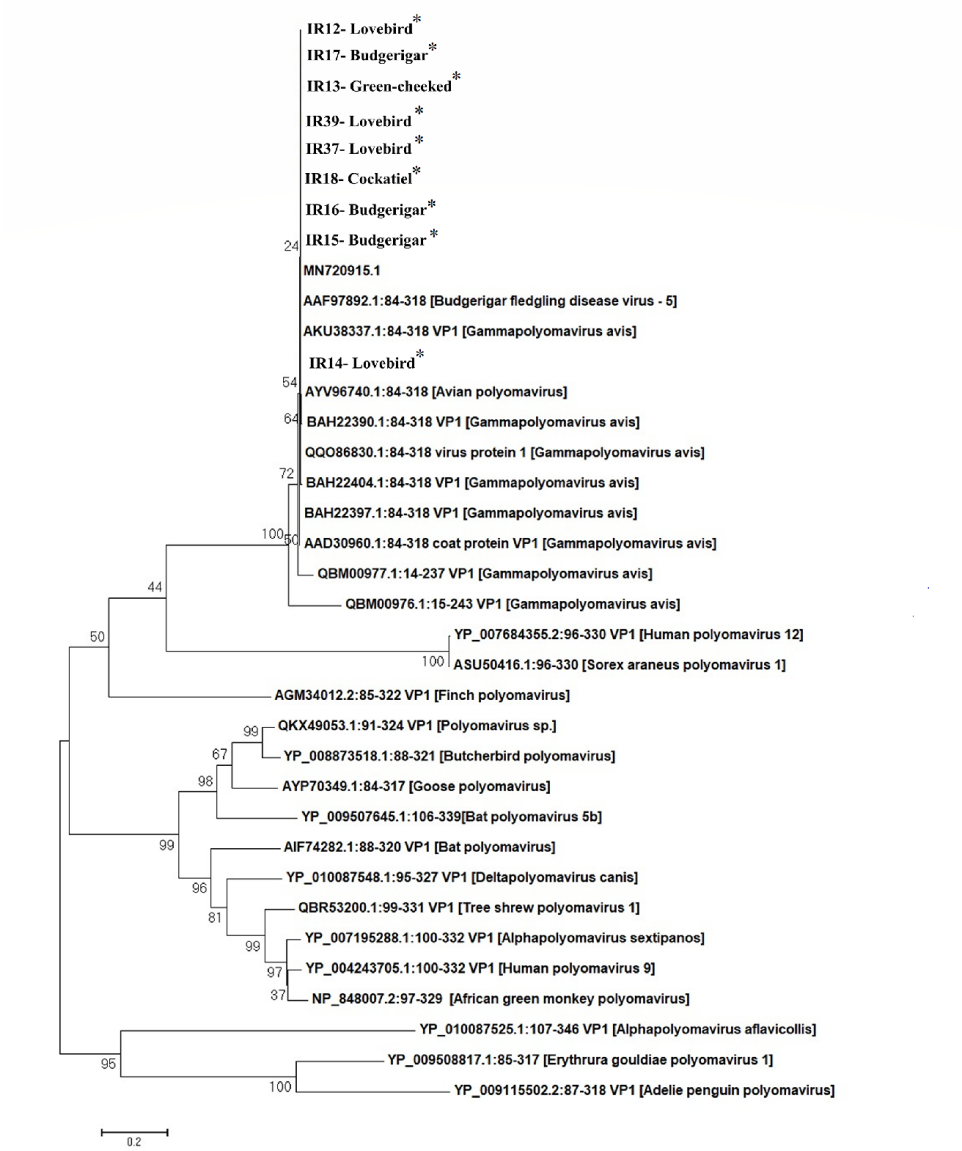

Fig. 5. Constructed phylogenetic tree based on deduced amino acid sequence of the VP1 gene. The samples analysed in this study are indicated with an asterisk (*) symbol.

tive on cloacal swabs. In general, a highly sensitive PCR method may provide more concordance between blood and cloacal samples of birds, making it useful for screening purposes (Phalen, 1998). Unfortunately, in the current study, we did not have access to the blood samples of the birds.

This study utilised a two-step amplification method for the detection of the

partial *VP1* gene of avian polyomavirus in suspicious samples, which involved an initial amplification round using primers from a previous study by Altan *et al.* (2016), followed by a second amplification round using primers designed in this study. By employing the two-step nested PCR approach, the virus was successfully detected in cloacal swab samples. This two-step amplification method was found to be more sensitive compared to other one-step amplification methods. In fact, when three samples tested negative in the first round, a positive result was still achieved in the second round, thereby demonstrating the increased sensitivity of the nested PCR method.

Previously, polyomavirus has been studied in young parrots of Pakistan. The mortality rate of birds infected with this virus in Pakistan was reported to be 90%. It has also been shown that the APV strain of Pakistani parrots was similar to those previously reported in other parts of the world, indicating a high frequency of APV infection in Pakistani parrots (Riaz *et al.*, 2019).

In this research, for the first time, the presence of APV was determined in the flocks of cockatiels, rosy-faced lovebirds, green-cheeked parakeets, and budgerigars in Iran. In this regard, referred birds to multiple bird clinics with feather/wing abnormalities were selected. Out of the 85 samples obtained from 5 different cities of Iran, 10 were positive. In general, 11.76% of birds were diagnosed positive. The observed incidence rate was greater than the reported numbers for Chile (1.6%), and Italy (0.79%), but lower than the rates reported for Taiwan (16.56%) and Turkey (23.0%) (Bért *et al.*, 2005; Thongchan *et al.*, 2016; González-Hein *et al.*, 2019; Adiguzel *et al.*, 2020). The results of nucleotide sequencing and phylogenetic analysis showed that the studied rosy-faced lovebirds are currently affected by at least three different strains of APV, while budgerigars are affected by two strains. With a mutation rate of 0.98% in the amplified region of the *VP1* gene, it is highly likely that there are additional strains of this virus that are currently circulating in Iranian birds and should be recognised by amplifying other parts of the virus genome.

A recently published study on the APV virus in Namibia revealed that all 11 sequenced specimens analysed in the study showed a close relationship with APV strains isolated from the genus *Psittacula* in China and South Korea. Meanwhile, some strains studied in the present study were placed in a separate cluster compared to Chinese and Korean strains (Molini *et al.*, 2023). Another research conducted in South Korea and published in 2023 revealed the presence of eight distinct APV strains circulating within the country. Among these strains, seven were classified within a shared genogroup alongside strains isolated from birds in America and Europe. Additionally, one strain was grouped within the genogroup of Asian strains, specifically those isolated from China and Korea (Yun *et al.*, 2023). In another study, three strains of the virus were identified in Brazilian Psittaciformes. Among these strains, two formed a distinct cluster similar to the findings in the present study, while one strain was grouped together with Chinese and European strains (Philadelpho *et al.*, 2022).

According to a published study investigating the prevalence of this disease in Turkey, it was found that the circulating strains in Turkey can be classified into four distinct clusters. Some of these strains were grouped together with European strains (specifically from Germany),

while others formed a cluster with Asian strains (Japan). Additionally, certain strains were found to be closely related to strains isolated from North America, while others formed a separate cluster. These findings are in accordance with the results of our study, indicating a global distribution of APV strains (Altan *et al.*, 2016). Investigation of Pakistani strains of APV in another research demonstrated that the three sequenced samples exhibited a significant similarity, with over 99% matching nucleotide sequences, to sequences documented in various regions across the globe. Similarly, in the current study, a notable similarity was observed between the sequenced samples from Iran and other circulating strains worldwide (Riaz *et al.*, 2019).

According to previous findings, there is no geographic separation for polyomavirus, as evidenced by the presence of hosts from Asia, North America, Europe, and Australia in the constructed phylogenetic tree of this study (Fig. 3). Reports from around the world, including this research, suggest that APV has a global distribution and may be transmitted through bird migration, the trade of ornamental birds, transportation of infected birds, and other means (Johne & Müller, 2007; Adiguzel *et al.*, 2020).

In light of the similarities in clinical symptoms between PBFDV and APV infections, it is recommended that the prevalence of mixed infection of these two viruses in ornamental birds of Iran should be examined in the future.

In conclusion, this study introduced a two-step PCR protocol for detecting APV via partial *VP1* gene in bird faeces. The results of this method confirmed the presence of APV in the cloacal and stool samples of the studied parrots. Furthermore, the phylogenetic analysis indicated that different strains of APV are currently circulating in ornamental birds of Iran.

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REFERENCES

Adiguzel, M. C., M. O. Timurkan & S. Cengiz, 2020. Investigation and sequence analysis of avian polyomavirus and psittacine beak and feather disease virus from companion birds in eastern Turkey. *Journal of Veterinary Research*, **64**, 495–501.

Altan, E., E. Eravci, U. Y. Cizmecigil, E. Yildar, O. Aydin, N. Turan, S. Ozsoy, K. B. Tekelioglu, T. Kurt, K. Gerbaga Ozsemir, E. Altan & H. Yilmaz, 2016. Detection and phylogeny of beak and feather disease virus and avian polyomavirus in psittacine pet birds in Turkey. *Journal of Exotic Pet Medicine*, **25**, 280–287.

Arnaout, R., R. A. Lee, G. R. Lee, C. Callahan, A. Cheng, C. F. Yen, K. P. Smith, R. Arora & J. E. Kirby, 2021. The limit of detection matters: The case for benchmarking severe acute respiratory syndrome coronavirus 2 testing. *Clinical Infectious Diseases*, **73**, 3042–3046.

Bért, E., L. Tomassone, C. Peccati, M. G. Navarrete & S. C. Sola, 2005. Detection of beak and feather disease virus (BFDV) and avian polyomavirus (APV) DNA in psittacine birds in Italy. *Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health*, **52**, 64–68.

Enders, F., M. Gravendyck, H. Gerlach & E. F. Kaleta, 1997. Fatal avian polyomavirus infection during quarantine in adult wild-caught red-faced lovebirds (*Agapornis pullaria*). *Avian Diseases*, **41**, 496–498.

González-Hein, G., I. A. Gil, R. Sanchez & B. Huaracan, 2019. Prevalence of Aves polyomavirus 1 and beak and feather disease virus from exotic captive psittacine birds in Chile. *Journal of Avian Medicine and Surgery*, **33**, 141–149.

Johne, R. & H. Müller, 2007. Polyomaviruses of birds: Etiologic agents of inflammatory diseases in a tumor virus family. *Journal of Virology*, **81**, 11554–11559.

Katoh, H., H. Ogawa, K. Ohya & H. Fukushi, 2010. A review of DNA viral infections in psittacine birds. *Journal of Veterinary Medical Science,* **72**, 1099–1106.

Lafferty, S. L., A. M. Fudge, R. E. Schmidt, G. Van Wilson & D. N. Phalen, 1999. Avian polyomavirus infection and disease in a green aracaris (*Pteroglossus viridis*). *Avian Diseases*, **43**, 577–585.

Latimer, K. S., F. D. Niagro, R. P. Campagnoli, B. W. Ritchie, D. A. Pesti & W. L. Steffens, 1993. Diagnosis of concurrent avian polyomavirus and psittacine beak and feather disease virus infections using DNA probes. *Journal of the Association of Avian Veterinarians*, **7**, 141–144.

Ma, J., R. Wu, Y. Tian, M. Zhang, W. Wang, Y. Li, F. Tian, Y. Cheng, Y. Yan, J. Sun, 2019. Isolation and characterization of an Aves polyomavirus 1 from diseased budgerigars in China. *Veterinary Microbiology*, **237**, 108397.

Moens, U., S. Calvignac-Spencer, C. Lauber, T. Ramqvist, M. C. W. Feltkamp, M. D. Daugherty, E. J. Verschoor & B. Ehlers, 2017. ICTV virus taxonomy profile: Polyomaviridae. *Journal of General Virology*, **98**, 1159–1160.

Molini, U., M. De Villiers, L. De Villiers, L. M. Coetzee, E. Hoebes, S. Khaiseb, G. Cattoli, W. G. Dundon & G. Franzo, 2023. Investigation and sequence analysis of psittacine beak and feather disease virus and avian polyomavirus from companion birds in Windhoek, Namibia. *Acta Tropica*, **238**, 1–8.

Phalen, D. N., 1998. Avian Polyomavirus: My Thoughts... *AFA Watchbird*, **25**, 28–39.

Philadelpho, N. A., R. D. Chacón, A. J. Diaz Forero, M. B. Guimarães, C. S. Astolfi-Ferreira & A. J. Piantino Ferreira, 2022. Detection of aves polyomavirus 1 (APyV) and beak and feather disease virus (BFDV) in exotic and native Brazilian Psittaciformes. *Brazilian Journal of Microbiology*, **53**, 1665–1673.

Reavill, D. R. & G. Dorrestein, 2018. Psittacines, coliiformes, musophagiformes, cuculiformes. In: *Pathology of Wildlife and Zoo Animals*, Academic Press, pp. 775–798.

Riaz, A., A. Yousaf, M. Moaeen-ud-Din, M. A. A. Shah, T. Zainab, S. Masood, N. Akhter & A. Ali, 2019. First detection and molecular characterization of avian polyomavirus in young parrots in Pakistan. *Veterinary Research Communications*, **43**, 197–202.

Ritchie, B. W., K. S. Latimer, J. Leonard, D. Pesti, R. Campagnoli & P. D. Lukert, 1998. Safety, immunogenicity, and efficacy of an inactivated avian polyomavirus vaccine. *American Journal of Veterinary Research*, **59**, 143–148.

Saribas, A. S., P. Coric, S. Bouaziz & M. Safak, 2019. Expression of novel proteins by polyomaviruses and recent advances in the structural and functional features of agnoprotein of JC virus, BK virus, and simian virus 40. *Journal of Cellular Physiology*, **234**, 8295–8315.

Ströh, L. J., N. H. Rustmeier, B. S. Blaum, J. Botsch, P. Rößler, F. Wedekink, W. Ian Lipkin, N. Mishra & T. Stehle, 2020. Structural basis and evolution of glycan receptor specificities within the polyomavirus family. *MBio*, **11**, e00745-20.

Thongchan, D., Y.-L. Huang, R. Khatri-Chhetri, S.-S. Tsai & H.-Y. Wu, 2016. Prevalence and pathology of avian polyomavirus (APV) and psittacine beak and feather disease virus (PBFDV) infections in Taiwan: A retrospective study

from 2010 to 2014. *RMUTI Journal*, **9**, 1–13.

Topor, S. V., 1999. Editorial: The control and prevention of avian polyomavirus infection. *Journal of Avian Medicine and Surgery*, **13**, 157–159.

Yun, Y. J., H. S. Song, Y. K. Kwon, C. K. Park & H. R. Kim, 2023. Genetic characterization of avian polyomaviruses identified from psittacine birds in South Korea. *Avian Pathology*, **52**, 420–425.

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