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Current situation of psittacine beak and feather disease virus in parrots

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ABSTRACT:

As a highly contagious disease that affects both wild and captive psittacine populations, psittacine beak and feather disease (PBFD) is caused by the beak and feather disease virus (BFDV). 170 tested parrots of 11 different species, blood and feather samples were taken from each parrot, these were then divided into three groups: diseased, suspected, and appeared healthy. Out of 340 samples, 147 samples showed amplification bands that could be seen, with a product size of 603 bp. There were various species categorized by The International Union for Conservation of Nature (IUCN 2019) based on Status. To examine the genetic sequence and phylogenetic tree, four of the eleven species (Amazona farinose, Ara ararauna, Psittacus erithacus, and Cacatua sulphurea) were selected. They are economically valuable, have varying continental origins around the world, and are most susceptible to extinction, which makes them important. In the phylogenetic analysis of OP831995 isolated from Cacatua Sulpher, OP831996 isolated from Amazon, and OP831997 isolates from Macaw, significant bootstrap with (AB1) as (MK803401 and MG257487) were observed. The partial sequences for four species were submitted to GenBank from other countries. additional noteworthy bootstrap using (N2), which corresponds to the isolates of African Gray parrots found in Egypt (OP831998 and OP831999) and the Kingdom of Saudi Arabia (MK803400 and MK803403). These findings suggested that mixed infections of different strains of the virus may be the primary cause of the acceleration of virus recombination, which could lead to the emergence of new strains that may have different host specificities.

INTRODUCTION

The most prevalent and deadly viral disease affecting parrots is called Psittacine Beak and Feather Disease (PBFD) **(Bert et al. 2005; Harkins et al. 2014; Raidal and Peters, 2018).** In Australia, it was initially documented

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in the early 1980s **(Pass and Perry, 1984).** Nowadays, it impacts a large variety of psittacine parrot species across the globe, affecting both wild and captive populations **(Fogell et al. 2016).** Because of BFDV's high environmental persistence and capacity to switch between closely related host species, PBFDV has become a major cause for concern for aviculturists and conservationists worldwide, spreading quickly **(Raidal SR; Cross GM, 1994 and Peters A et al. 2014).**

The beak and feather disease virus (BFDV) aetiological agent is a member of the genus Circo, family Circoviridae, which includes 49 species **(Rahaus M and Wolff, 2003).** It's a virus made of single-stranded DNA (ssDNA). It has a negative sense genome organization and is a closed circular DNA genome with 1.7–2.3 kilobases **(Ritchie B.W et al. 1990, 1988**). Two major open reading frames (ORFs) oriented in the opposite direction (ambisense) are present in the genomes of viruses in the genus Circovirus. One ORF encodes the replicase protein (Rep) ORF V1, and the other encodes the immunogenic capsid protein (Cap) ORF C2 **(Hess M et al. 2004).** The virus's replicative form has seven putative ORFs, according to an analysis of it. In the virion strand, three of them were recognized, and four ORFs were found in the replicative strand. These ORFs potentially encode viral proteins (**Rahaus M and Wolff (2003))**.

According to **Albertyn et al. (2004); De Kloet and De Kloet (2004) and Khalesi et al. (2005),** the virus isolates should be grouped according to the host species or the virus's infectious potential rather than their geographic location**. Shearer et al. (2009)** provided support for this theory by demonstrating that the BFDV virus isolated from cockatiels differed from other isolates both genetically and serologically. However, strains of the virus from Southern Africa have divided into lineages that are geographically distinct from viruses that are found globally **(Heath et al. 2004).**

Circoviruses cause long-term immunological suppression as well as abnormalities in the feathers and beaks of birds. BFDV is a serious concern to aviculturists due to its rapid global spread. It has the ability to switch between hosts that are closely related. According to **Ritchie et al. (1991),** BFDV can spread quickly through contact with contaminated feather mud, surfaces, or objects (horizontal mode). However, in a vertical mode, the virus can even be passed directly from a female to her offspring **(Raidal SR et al. 1993).** Further evidence that these species could be virus carriers comes from the detection that it is prevalent in non-psittacine species (**Amery-Gale et al. 2017 and Sarker et al. 2016**).

Numerous optimized diagnostic tests for BFDV screening have been carried out and have significantly improved since its initial scientific evaluation. Currently, the most popular assay for BFDV antibody detection is haeminhibition assay. According to **Eastwood et al. (2015),** it is widely appropriate for the detection of a significant percentage of psittacine species and does not require a secondary antibody. In contrast to methods that depend on gel detection, probe-based assays are able to detect viral DNA at much lower concentrations, which is why quantitative (real-time) PCR techniques are increasingly being used to ascertain the viral concentration **(Johanne et al. 2020).**

However, reagents and equipment needed for standard PCR screening are less costly than those needed for probe-based assays, and as a result, it is likely that this method will continue to be widely used for BFDV screening in general. Commonly used primers are those found in ORF V1, which codes for the replication-associated protein. Although homology studies of various ORF V1 fragments obtained from BFDV isolates from different species of birds have suggested the existence of species-specific lineages of viruses, this region was found to be highly conserved **(Ritchie et al. 2003).** However, nucleotide sequences from the less well-conserved capsid gene ORF C1 do not confirm these findings **(Raue et al. 2004).**

Circoviruses exhibit high rates of mutation (nearing that of RNA viruses) and an unusually diverse genome sequence composition. Given that the Rep protein is a genetically conserved

sequence, the ORF-V1 gene is more likely to have a conserved primer design than the capsid protein. Conserved primers enable PCR-based technologies to identify the majority of isolates despite the diversity of BFDV genotypes **(Bassami et al. 2001 and Ritchie 2003).** In the current study, the spread of the virus to Egypt is verified by PCR and sequencing. This study's primary objective was to use gene sequencing analysis to evaluate the diversity and possible recombination events of this virus. However, some deaths with clinical indications of this virus were documented. Accordingly, stringent procedures for examining imported parrots and regular checks of the existing parrot farm are deemed necessary preventive steps to stop the virus's spread **(José L, Teel et al. 2022).**

MATERIALS and METHODS

Sampling:

During 2022 and 2023, three hundred forty feather follicles and blood samples were collected from 11 species. Blood samples were collected from the brachial vein and stored in absolute ethanol for molecular analysis. The samples were provided by private breeders. Sixty-four birds that showed clinical signs of PBFD were collected. These symptoms include the bird becoming less active and vibrant, losing and deforming feathers, and developing certain secondary diseases like fungi and bacteria as a result of viral infection **Figure (1)**. According to Positive PBFD virus Cases, we collected samples from fifty-three birds in contact with diseased cases. And fifty-three birds were collected from heathy cases, **Table (1)**.

Figure 1: An injury to an African gray parrot is depicted in Picture A, along with the appearance of skin exposed due to the bird's loss of feathers in the chest and wings. Regarding the cockatoo parrot in image B, its legs and chest have completely lost their feathers, leaving only malformed feathers visible

Polymerase chain reaction (PCR):

Extraction of DNA:

The DNA easy blood and tissue kit QIAGEN Cat. No, 69504 and 69506 were used to extract DNA from 170 blood samples and 170 feather samples respectively.

Blood sample.

100 µl each sample of anticoagulated blood adjust till 220 µl with PBS and 20 µl proteinase K were added.

Tissue sample.

2–5 mm of feather fragments was cut and transferred into sterile 1.5 mL Eppendorf tubes. For each sample, a new blade was used.

In brief, the procedure was followed as directed by the manufacturer: 180 µl ATL buffer and 20 µl of proteinase K were added to the samples then Incubated at 56ºC for 2 hours. Next, 200 µl AL buffer was added to samples and placed at 70 ◦C for 5 min. After that 200 µl ethanol was added. Subsequently, the lysate was transferred onto the spin columns which supplied by the kit, and the tubes were centrifuged. DNA bound to the resin was washed twice with AW1 and AW2 buffer. Ultimately, DNA was eluted with 50 µL. The purified DNA was stored at −20 ◦C. The concentrations of extracted DNA were measured with SPECTRO star Nano (BMG LABTECH, Ortenberg, Germany).

PCR amplificaion

Rep genes were amplified by PCR, according to Ritchie et al.,2003. PCR was carried out with primers Table 2 synthesized at Sigma (Welwyn Garden City, UK). The PCR mix contained double distilled water, 10 µl AmpliTaq Gold Fast PCR Master Mix, (Thermos Fisher Scientific) Cat. no. MAN0009870, Waltham, MA, USA),0.2 µM of each primer and 5 µL of DNA template or water for no template control (NTC). PCR was performed according to Table 2. The products of the PCR reactions were separated by electrophoresis in 1.5% agarose containing 10 g/ml ethidium bromide and viewed under UV light.

Table 1. Details of samples collected from different parrots' species IUCN red list of threatened species

Family	Genus and Species	Common name	Status by IUCN 2019	Number of birds	Apparently healthy	Diseased	Suspected cases
Psittacidae	Ara ararauna	Blue and gold Macaw	Near threatened	5	2	2	1
	Amazona farinose	Mealy amazon	Near threatened	3	1	1	1
	Amazona aestiva	blue front amazon	Least concern	5	1	2	2
	Aratinga solstitialis	Sun parakeet (sun conure)	endangered	20	5	10	5
	Myiopsitta monachus	Quaker parrot	Least concern	20	5	5	10
	Poicephalus senegalus	Senegal parrot	Least concern	20	5	10	5
	Psittacus erithacus)	African grey parrot	Endangered	20	10	5	5
Cacatuidae	Nymphicus hollandicus	Cockatiel	Least concern	30	10	10	10
	Cacatua sulphurea	yellow-crested cockatoo	Critically endangered	5	2	2	1
Psittaculidae	Agapornis fischeri	Love birds	Least concern	22	7	7	8
	Psittacula krameria	Alexandrine parakeet	Near threatened	20	5	10	5
Total samples				170	53	64	53

Table 2. oligonucleotide sequence target genes, amplicon sizes, and cycling conditions used in this study

Primers sequences	Amplified segment (bp)	Amplification (40 cycles)			Reference
		Denaturation	Annealing	Extension	
5−TACACCTACAGAC GGCGA−3 5−GGCGGAGCATCTC GCATAG−3	603	96^{0}C /3 sec	54.5^{0}C /3 sec	68^{0}C/15 sec	Ritchie et al., 2003

Partial Sequencing of *rep* gene:

the DNA templates were Sequenced by Sanger dideoxynucleotide sequencing after PCR product purification using BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher, USA) according to the manufacturer's instructions. A 3.2 pmol of reverse primers was applied. The sequencing products were purified by using Centri-Sep™ Spin Columns (Thermo Fisher, USA), and injection on capillary electrophoresis systems 3500 Genetic analyzers (Applied Biosystems, USA) was done. Sequence analysis.

The Sequences were assembled. Multiple nucleotides and predicted amino acid sequence alignment were performed using the Clustal W **(Chenna et al. 2003)** algorithm in the BioEdit software version 7.1 **(Hall, 1999)**. in MEGA11 software, the sequences were aligned **(Tamura et al. 2021).**

The identity percentages of the obtained sequences were spotted using the GenBank using the alignment tool of Basic Local Alignment Search Tool (BLAST) (https://blast. ncbi. nlm. nih.gov/Blast.cgi). Phylogenetic trees were constructed for partial rep gene sequence. Phylogenetic trees were inferred using the Maximum Likelihood approach implemented within MEGA11 software, and the topology was estimated by bootstrapping over 1000 replicates **(Saitou and Nei, 1987) and (Tamura et al. 2004, 2021)**.

RESULTS

In this study, 170 parrots from 11 different species were used, and 340 samples which include feather and blood samples, were obtained from each bird. **Table (1)** displays the cases that were classified as Diseased, Suspected, and Healthy. 147 of the 340 BFDV samples that were examined exhibited distinct amplification bands, with a product size of 603 bp, as shown in Table (2). 87 of these samples tested positive for blood, and the remaining 60 samples tested positive for feathers, 83 samples of blood and 110 samples of feathers yielded negative results. The results also revealed that the highest infection rate was found in diseased cases (23.5%), followed by seemingly healthy cases (13.5%) of all samples. The infection rate was 1.6% in suspected birds. as shown in **Table (3).**

Table 3. Details of positive and negative PBFDV samples in both blood (B) and feathers (F) Collected from tested parrot species

Species	Number of samples	Positive samples		Apparent Healthy		Diseased		Suspected cases		Negative samples	
		B	F	B	F	B	F	B	F	B	F
Ara ararauna	10	2	2	0	0	2	2	0	0	3	3
Amazona farinose	6	3	1	1	0	1	1	1	0	0	2
Amazona aestiva	10	3	2	1	0	2	2	0	0	2	3
Aratinga solstitialis	40	8	5	4	1	4	4	0	0	12	15
Myiopsitta monachus	40	6	4	4	2	2	2	0	0	14	16
Poicephalus senegalus	40	15	10	5	2	7	6	3	2	5	10
Psittacus timneh	40	12	7	6	2	5	5	1	0	8	13
Nymphicus holland-	60	13	11	4	3	6	5	3	3	17	19
Cacatua sulphurea	10	3	2	1	0	2	2	0	0	2	3
Agapornis fischeri	44	12	7	5	0	5	5	2	2	10	15
Psittacula krameria	40	10	9	3	2	5	5	2	2	10	11
Total	340	87	60	34	12	41	39	12	9	83	110

DNA sequence and sequence analysis:

Isolates Amplified sequence subjected for BLASTn alignments applied with sequences of those closely related species in same taxa in GenBank database. The partial-length *rep* gene of the isolate accession number OP831995, OP831996 and OP831997, OP831998, OP831999 were amplified and sequenced, yielding a 603-bp fragment, nucleotide sequence accession numbers: All nucleotide sequences detected in this study have been submitted to GenBank under the accession numbers listed in **Table (4)**.

Table 4. Gene bank accession number with different species.

Common name	Scientific name	Accession	Status by IUCN 2019
yellow-crested cockatoo	Cacatua sulphurea	OP831995	Critically endangered
Mealy amazon	Amazona farinose	OP831996	Near threatened
Blue and gold Macaw	Ara ararauna	OP831997	Near threatened
African grey	Psittacus timneh	OP831998	Endangered
African grey	Psittacus timneh	OP831999	Endangered

Phylogenetic tree:

was created using the partial sequence of rep gene deduced amino acid. conducted in MEGA11 **(Tamura et al. 2021)**, using the Maximum Likelihood method and the Tamura-Nei model **(Tamura and Nei, 1993).** The tree with the highest log likelihood (-1930.82) is shown. The colour blue represents the BFDV strain isolated in this study. Gen Bank IDs are located in each sequence, Initial tree(s) for the heuristic search were obtained automatically by applying NeighborJoin and BioNJ algorthma to a matrix of pairwise distances estimated using the JTT model and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analysis was conducted in MEGA11, shown in **Figure 2 (Tamura et al. 2021).** It was found that there are two types of the virus) PBFD-AB1 and PBFD -N2) that are identical to the country close to Egypt, which is the Kingdom of Saudi Arabia**.** The percentages of similarity (identity matrix) and difference (diversity matrix) between the strains found in Egypt and the neighbouring countries were also displayed in the results. **Figure 3.**

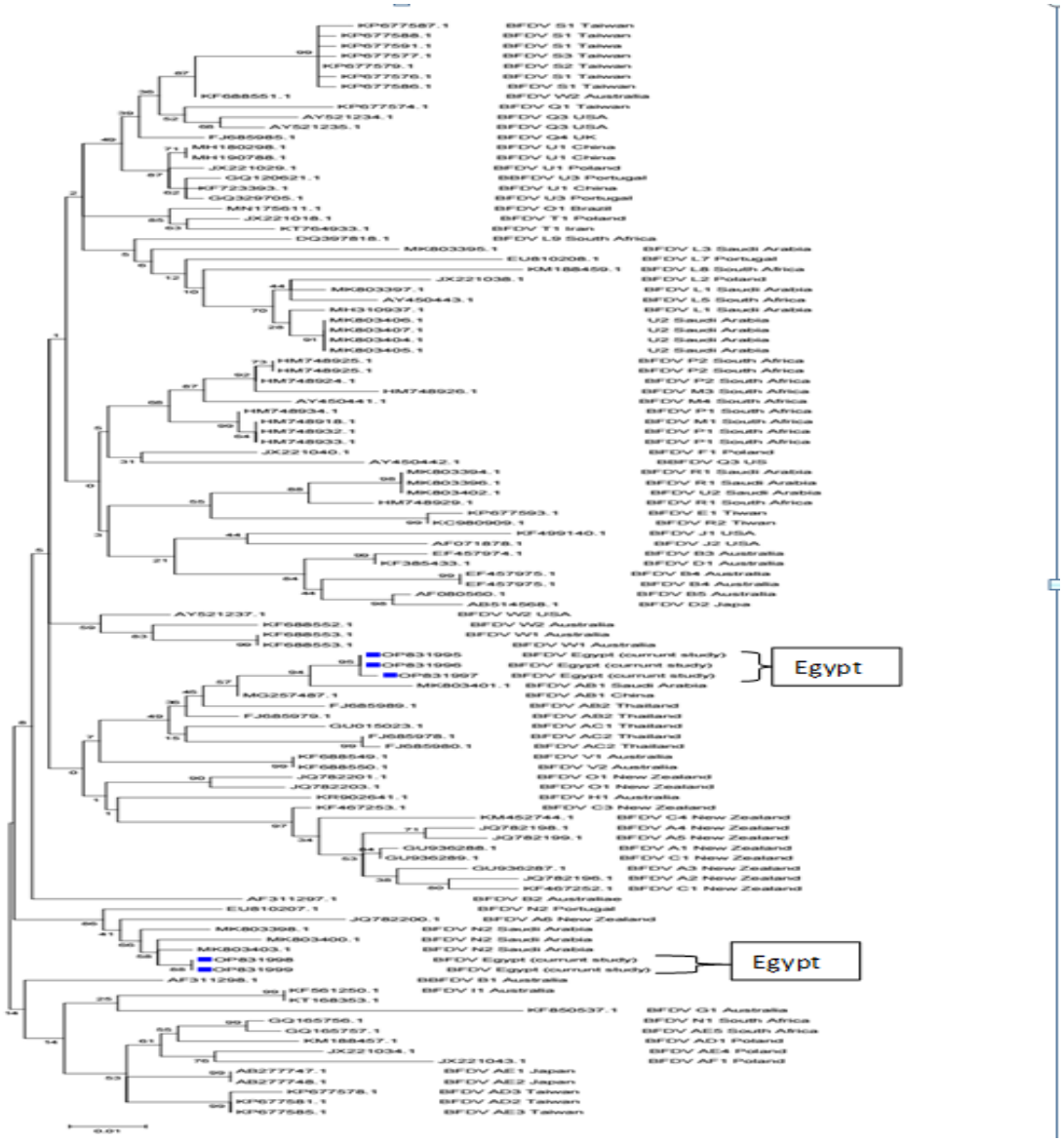
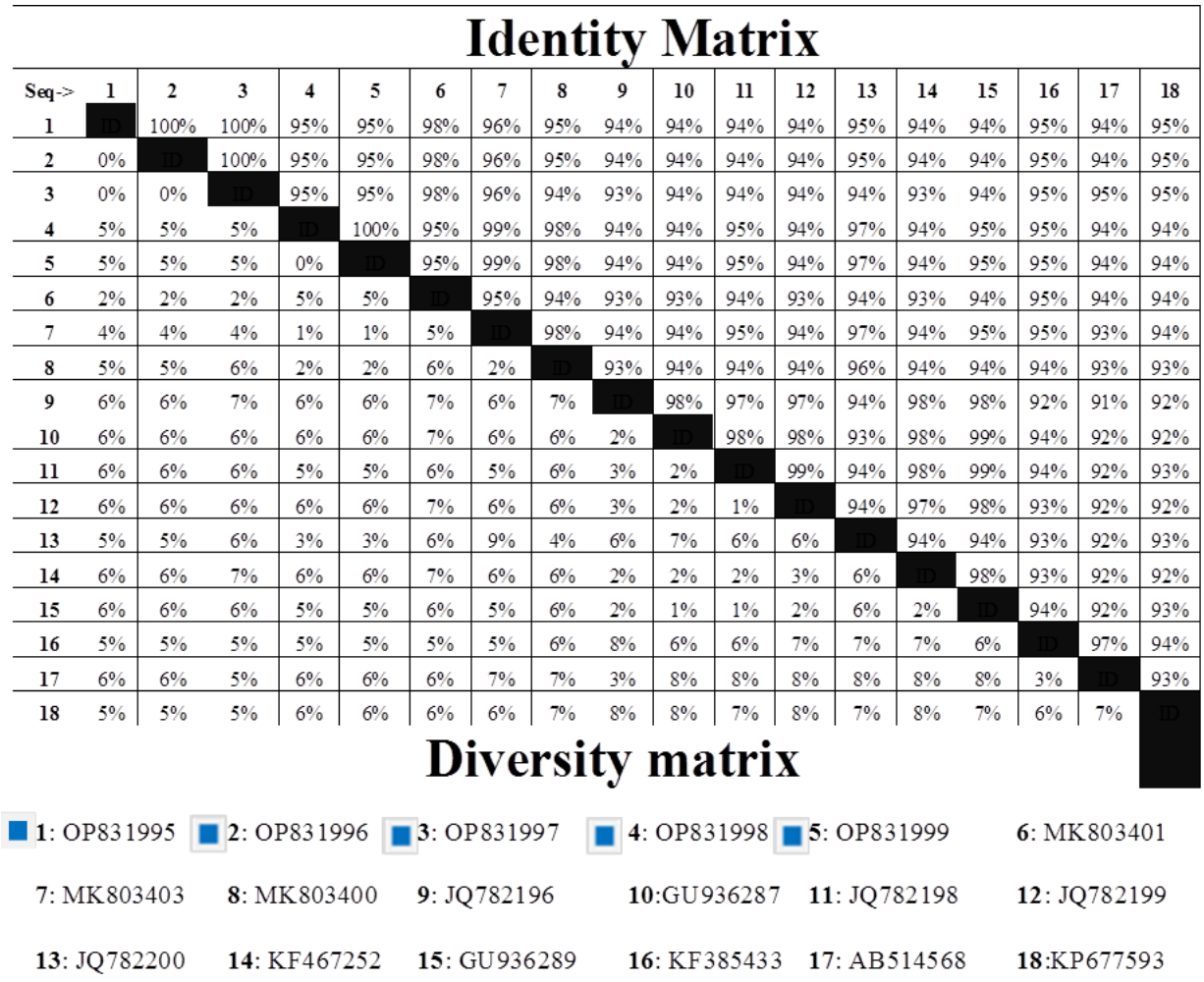


Figure 2. Phylogenetic tree of studied BFDV compared with other circulating strains.



Identity Matrix

Seq->	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	ID	100%	100%	95%	95%	98%	96%	95%	94%	94%	94%	94%	95%	94%	94%	95%	94%	95%
2	0%	ID	100%	95%	95%	98%	96%	95%	94%	94%	94%	94%	95%	94%	94%	95%	94%	95%
3	0%	0%	ID	95%	95%	98%	96%	94%	93%	94%	94%	94%	94%	93%	94%	95%	95%	95%
4	5%	5%	5%	ID	100%	95%	99%	98%	94%	94%	95%	94%	97%	94%	95%	95%	94%	94%
5	5%	5%	5%	0%	ID	95%	99%	98%	94%	94%	95%	94%	97%	94%	95%	95%	94%	94%
6	2%	2%	2%	5%	5%	ID	95%	94%	93%	93%	94%	93%	94%	93%	94%	95%	94%	94%
7	4%	4%	4%	1%	1%	5%	ID	98%	94%	94%	95%	94%	97%	94%	95%	95%	93%	94%
8	5%	5%	6%	2%	2%	6%	2%	ID	93%	94%	94%	94%	96%	94%	94%	94%	93%	93%
9	6%	6%	7%	6%	6%	7%	6%	7%	ID	98%	97%	97%	94%	98%	98%	92%	91%	92%
10	6%	6%	6%	6%	6%	7%	6%	6%	2%	ID	98%	98%	93%	98%	99%	94%	92%	92%
11	6%	6%	6%	5%	5%	6%	5%	6%	3%	2%	ID	99%	94%	98%	99%	94%	92%	93%
12	6%	6%	6%	6%	6%	7%	6%	6%	3%	2%	1%	ID	94%	97%	98%	93%	92%	92%
13	5%	5%	6%	3%	3%	6%	9%	4%	6%	7%	6%	6%	ID	94%	94%	93%	92%	93%
14	6%	6%	7%	6%	6%	7%	6%	6%	2%	2%	2%	3%	6%	ID	98%	93%	92%	92%
15	6%	6%	6%	5%	5%	6%	5%	6%	2%	1%	1%	2%	6%	2%	ID	94%	92%	93%
16	5%	5%	5%	5%	5%	5%	5%	6%	8%	6%	6%	7%	7%	7%	6%	ID	97%	94%
17	6%	6%	5%	6%	6%	6%	7%	7%	3%	8%	8%	8%	8%	8%	8%	3%	ID	93%
18	5%	5%	5%	6%	6%	6%	6%	7%	8%	8%	7%	8%	7%	8%	7%	6%	7%	ID

Diversity matrix

Figure 3: Percentage of identity and diversity matrix between selected samples of newly circulating BFDV and the strains obtained through this study (blue mark).

DISCUSSION

The clinical signs of infected birds from which feather samples were taken showed that this disease develops in parrots according to the age of the bird, while smaller birds have a faster progression of the disease. This chronic disease is the most common and is characterized by a symmetrical, slowly progressive atrophy of the developing feathers that gets worse with each successive replacing of feathers. These parrots are often immunocompromised and die from bacterial, fungal, or other viral infections **(Fogell D J et al. 2018).**

All positive results of the PBFD virus have feather and beak abnormalities, and when swiping your hand between feathers shouldn't have dust. PBFD reduces the amount of feather dust produced because the contour feathers are not normal. Beak and foot colours are changed while emerging feathers are small, twisted, and very abnormal and some feathers lack colour. Developing feathers normally close off blood supply when mature while Feathers affected by BFDV do not close off or are fractured and dried blood can be seen in the calamus. Deformed beaks and all tail feathers are missed, and after replacing feathers new feathers do not grow **(Jackson B et al. 2014).**

Although PBFDV infection is thought to be possible in all species of parrots, it is most common in birds under three years old and is primarily observed in those with African and Australasian origins. The lymphoid tissues of the gastric canal, including the bursa of Fabricius, appear to be PBVDV portals of entry. Prior to the virus spreading secondary to the liver, thymus, epidermis, and other tissues, primary replication of the virus takes place in these intestinal lymphoid organs **(Helene Pendl, 2016)**

El Shahidy M et al.,(2018) reported that Egypt's native psittacine species are susceptible to PBFD, and they neglect to take into account how BFDV may affect both domestic and imported captive parrot populations. The results indicate that there is no appreciable difference in the prevalence of circovirus between Egyptian birds kept in captivity and birds imported. The investigation's findings indicate that, of the 340 samples tested, the mean circovirus result was comparatively high, with (147) samples testing positive. Blue front Amazon with yellow-crested cockatoos (50%) and Mealy Amazon parrots (66%), Senegal parrots (62.5%) The species with the highest prevalence among those tested were African Grey Parrots with Psittacula krameria parrots (47.5%), Love Birds (43.1%), and Blue Gold Macaw with Cockatiel Parrots (40%). were the species that, of those tested, had the highest prevalence. When all kinds of quaker parrots are considered, it ranks last (25%). The next highest percentage of birds that are imported from open wild places are heavily exploited for both the legal and illegal trapping and export of companion birds for the pet trade **(IUCN 2015).**

Considering that the genus Cacatua, which includes the yellow-crested cockatoo, was the source of the first description of PBFD, 11 of these species have been shown to be vulnerable to BFDV infection, particularly when imported from Southeast Asia, where many of these species are native. Bird specimens from both wild and captive environments have been found to be carrying the virus **(Ogawa H et al. 2013).**

Notably, one genus necessitates further investigations, Psittacula krameria which has populations spread across about 35 countries, is the most noticeable parrot globally **(Tayleur JR. 2010).** With a positive rate of 4% of all examined samples, the virus was also isolated from African Gray parrots in countries near Egypt, including the Kingdom of Saudi Arabia **(Alaudeen Hakami et al. 2017).** Lastly, some research suggests that some species might be less susceptible to contracting BFDV infection, such as cockatiels that own quaker parrots **(Shearer PL, et al. 2008).**

PBFD has become a major cause for concern to conservationists and aviculturist. . Recently, the virus has spread throughout the entire world. According to **Heath et al. (2004),** psittacine beak and feather disease is becoming more prevalent in Africa. Furthermore, international trade probably played a major role in

Saudi Arabia's introduction of BFDV **(Hakami et al. 2017and Mohamed et al. 2021).**

Cases in the UAE that were confirmed in 2016 **(Hakimuddin et al. 2016)** Research on BFDV has been screened, disseminated, and examined globally, with an emphasis on wild populations. Recent research has concentrated on examining the phylogenetic analysis, evolution, and viral recombination processes. Types of tissue utilized in screening in the capitative population, on the other hand, the most commonly used sources of samples were feathers (34.2%) and blood (32.5%).

According to the **IUCN (2019),** our study is the first attempt to identify BFDV in critically endangered parrot species found in Egypt. The amplification bands were distinguished for each species by a 603 bp product size. The genomes of the biggest and most economically valuable species have been sequenced; these species are traded globally, either as commodities or as captive animals kept in commercial or private zoos. This is due to the genetic sequencing is believed to be essential for distinguishing isolates from various global locations, such as Australia, South America, and Africa. Gene Bank was used to analyze the genetic sequence of each species. Using the deduced partial rep amino acid sequences of the five BFDV isolates under study and their accession numbers (OP831995, OP831996, OP831997, OP831998, and OP831999).

The presence of two distinct strains, BFDV N2 and BFDV AB1, was suggested by the results of the clustering of BFDV strains from Egypt with different groups that were submitted to GenBank from other countries. The first strain, BFDV N2, is similar to the African Gray parrot isolates discovered in Egypt (OP831998 and OP831999) and is comparable to isolates of the same kind found in the Kingdom of Saudi Arabia (MK803400 and MK803403).According to phylogenetic analysis, the second strain, BFDV AB1, showed significant bootstrap with MK803401 isolate AB1 isolated from Cacatua alba in Saudi Arabia, MG257487, as was OP831995 isolated from Cacatua Sulpher, OP831996 isolated from Amazon, and OP831997 isolates from Macaw. One AB1 isolate was discovered in China. These findings indicated that mixed infections of distinct strains, which led to the emergence of new strains with potential host specificity variations, were the primary cause of the acceleration of viral recombination **(Mahmoud M et al. 2021).**

On the other hand, to compare the remaining samples of this study with those of other positive species, it still be necessary to investigate their genetic sequences in the future, even if those species are not as economically valuable as this one. Since BFDV-N2 was found in African grey parrots, the host specificity by **Ritchie et al. (2003)** demonstrated that human breeding facilities with one or a small number of bird species may affect host specificities. The examined birds demonstrated that even in cases where a BFDV test is positive, certain birds may continue to show no symptoms (**Ritchie BW et al. 1989).** and to prevent the illness from spreading to other birds, screening birds who do not show symptoms is recommended (**Regnard GL et al. 2015)**. But imported birds should have vaccination certificate against this virus checked for regularly by the relevant veterinary authorities. The locations of migratory or wild birds found throughout Egypt must also be regularly monitored, in addition to the birds housed in zoos. We also recommend pet bird breeders clinically tested the beaks and feathers of their birds for any signs of the virus. Continuous surveillance should be carried out to monitor the status of PBFD virus in Egypt.

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Author contributions

Mohamed I. AbdAllah designed the study, Sara M. Elnomrosy and Omnia M. Khattab analyzed the data, Mohamed I. AbdAllah and Sara M. Elnomrosy contributed to writing, Naglaa M. Hagag and Momtaz A. Shahein critically reviewed the manuscript, and assisted in analysis of data. All authors performed the study and wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.