

Studies on Circo and Polyoma Viruses in Psittacine Birds in Egypt, During 2014-2016

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Abstract:

In recent years various psittacine birds have been popular and kept as pet animals in Egypt. *Circo* and *Polyomaviruses* and are the main causative agents of Psittisine beak and feather deformities. The main objectives of this study were centered on DNA virus infections in psittacine birds with special refreance to *Circovirus* and *Polyomaviruses*. Feather and tissue samples were collected from infected birds and examined using PCR based technology. A total of 50 infected Psittacine birds were examined in which feather and tissue sample were collected and screened for the presence *Circo* and *Polyomaviruses* by using PCR technology. PCR was used to amplify C1 gene of *Circovirus* and T gene of *polyomavirus* in extracted DNA. The highest prevalence of *Circovirus* infection in feather samples was shown in red rumped parakeet followed by budgerigar and love birds (41.66, 26.92 and 33.33; respectively). The distribution rate of *polyomavirus* infection in parakeet, budgerigar and love birds were 23 %, 23.7% and 16.66 % respectively..Co-infection with *Polyoma* and *Circovirus*, was detected in, three parakeet, six budgerigars and two love birds with a total percentage of 22%. Apparently healthy psittacine birds can be latently infected with either or both *Circo* and *Polyomavirus* without showing any clinical symptoms, and become carriers for and virus shedding disease transmission to other birds. In future studies in Egypt, an attempt to isolate *Circo* and *Polyoma* viruses from field cases should be done and the isolates could use for development of bivalent prophylactic vaccine for Egyptian psatticine birds.

Keywords: psittacine birds, polyomavirus, psittacine beak and feather virus, PCR.

Introduction:

In recent years various psittacine birds have been popular and kept as pet animals in Egypt. Infectious

diseases of psittacine birds especially viral diseases are the most common clinical problems in captive psittacine birds because of

difficulties in treatment and control and their association with acute death and deformities in peak and feathers (*Ritchie et al., 2003*).

Many viral infections in psittacine birds have been reported worldwide including DNA and RNA viruses. DNA virus infections associated with high morbidities and mortalities in psittacine birds including, psittacine beak and feather disease (PBFD) caused by *Circovirus* infection (*Pass and Perry, 1984*), avian *Polyomavirus* infection (*Bernier et al. 1981*), *psittacid Herpesvirus* infection (*Simpson et al., 1975*), *psittacine Adenovirus* infection (*Raue et al., 2005*), *Poxvirus* infection (*McDonald et al., 1981*) and *Papillomavirus* infection (*Cooper et al., 1986*). Concerning to *RNAvirus* infections in psittacine birds were including, *Reovirus* infection (*van den Brand et al., 2007*), *Coronavirus* infection (*Gough et al., 2006*), *Paramyxovirus* infection (*Grund et al., 2002*), *Influenzavirus* infection (*Pillai et al., 2008*) and *Bornavirus* infection (*Kistler et al., 2008*).

Circo and *Polyomaviruses* represent the most prevalent cause of feather and beak deformities in psittacine birds with two different disease syndrome. Psittacine beak and feather disease (PBFD) caused by *Circovirus* and Budgerigar fledgling disease (BFD) caused by avian *Polyomavirus*. PBFD is widely distributed in many countries of the world, as Australia (*Khalesi et al., 2005*). PBFD has been confirmed in

over 60 species of both free ranging and captive psittacine birds (*Todd, 2004*).

In Egypt information about the incidence and prevalence of beak and feather disease & Budgerigar fledgling disease in psittacine birds are poorly understand hence this study considered a preliminary to understand the virus causes of feather and beak losses and dystrophy that affect beak and feather of psittacine birds in Egypt. The main objectives of this study were centered on DNA virus infections in psittacine birds with respect to *Circovirus* and *Polyomaviruses* the main causative agents of Psittacine beak and feather deformities. To achieve this work, feather and tissue samples were collected from infected birds to evaluate the incidence of both viruses in psittacine birds in Egypt using PCR based technology and histopathology.

Materials and Methods:

Psittacine birds

Examined Thirty apparently healthy birds and twenty clinically affected dead birds suffering from loss of appetite, vomiting, diarrhea and feather deformities were collected. The examined birds included: 26 budgreigars, twelve red rumped parakeets, and twelve love birds. Dead birds were examined clinically and for post mortem lesions. Bird samples were collected over a period of three years starting from 2014 and ending early 2016 All samples were

collected from Port Said provinces at different localities.

Feather and tissue samples

Feathers and tissues from clinically affected dead birds and apparently healthy birds were collected. Feather samples were plucked from the chest area of juvenile and adult birds. Liver, spleen and intestine were collected from diseased birds

DNA extraction

DNA was extracted from feathers and tissues samples using QIA amp DNA minikit (Qiagen), Catalogue No.51304. QIA DNA minikit combines the selective binding properties of a silica gel based membrane according to manufacture instructions with the speed of the microcentrifuges.

PCR for Circovirus

PCR amplification of ORF1 fragment of 202 bp in length, as described by *Raue et al. (2004)*. The sequence of primers was as follows: P2-5'-AAC CCT ACA GAC GGC GAG-3' (182-199) and P4-5'-GTC ACA GTC CTC CTT GTA CC-3' (879-898) using the HotStar Taq® Plus Master Mix PCR kit (QIAGEN, Hilden, Germany). Each reaction tube contained 1 unit of HotStar Taq® Plus DNA polymerase in 1× PCR buffer containing (KCl and (NH₄)₂SO₄), 200uM of each dNTP, 0.5 μM of each of the forward and reverse primers, 1× CoralLoad dye mix, and 3 μl of the sample DNA and controls. Thermal cycling conditions for amplification of circoviral genes were: an initial denaturation step (95°C for 4 min), amplification

cycles (95°C for 30 sec, 54.5°C for 30 sec, and 72°C for 50 sec), and a final extension step (72°C for 5min).

PCR for Polyomavirus

PCR amplification of a 310 bp fragment of the APV genome was performed using primers described by by *Johne and Müller (1998)*. The sequence of primers was as follows: 5'-CAA GCA TATGTC CCT TTA TCC C-3'(4303-4324) and 5'-CTGTTT AAG GCC TTC CAA GAT G -3' (4,612-4,591).using the HotStar Taq® Plus Master Mix PCR kit (QIAGEN, Hilden, Germany). Each reaction tube contained 1 unit of HotStar Taq® Plus DNA polymerase in 1× PCR buffer containing (KCl and (NH₄)₂SO₄), 200uM of each dNTP, 0.5 μM of each of the forward and reverse primers, 1× CoralLoad dye mix, and 3 μl of the sample DNA and controls. Thermal cycling conditions for amplification of polya virus genes were: an initial denaturation step (95°C for 4 min), amplification cycles (95°C for 30 sec, 54.5°C for 30 sec, and 72°C for 50 sec), and a final extension step (72°C for 5min).

Sequencing and Phylogenetic analysis

Purified PCR products were sequenced in the foreword and reverse directions on an applied biosystem 3130 automated DNA sequencer (ABI 3130, USA) using a ready reaction Bigdye terminator V 3.1 cycle sequencing kits (Perkin Elmer/applied biosystem, Forster city, CA. Cat. No.4336817). The generated sequences were uploaded

to the gene bank to establish the similar sequences elsewhere all over the world. Blast analysis were done using Geneious software in comparison to the generated sequences. Strains identified were aligned with other strains obtained from gene bank using MAFFT alignment (Standly *et.al*, 2013). Comparative analysis of the sequences was performed using Clustal V multiple sequence alignment program, version 1.83 of MegAlign module of Lasergene DNA star software pairwise Phylogenetic tree were constructed using Mega 5.10 program with neighbor joining method (Tamura *et al.*, 2011).

Results

Amplification of C1 gene of *Circovirus* in psittacine birds using PCR

Total DNA extracted from 50 pooled feather sample and pooled tissue samples were used as template for amplification. The predicted band size for these primers were a PCR product with a size of 202 bp (Fig. 1). Total positivity percentage of *Circovirus* in pooled feather and skin samples (32%) is higher than pooled tissue samples (20%). The highest prevalence of *Circovirus* infection in feather samples was shown in red rumped parakeet followed by budgrigar and love birds with positivity % of 41.66 , 26.92 and 33.33 respectively.

Sequence analysis of C1 gene of Psittacine *Circovirus*

Nucleotide sequence

Sequencing and analysis of PCR-positive samples to *Circovirus* from red rumped parakeet in Egypt were amplified typical fragments of C1 gene of *Circovirus* genome. Analysis of the nucleotide sequence of Egyptian *Circovirus* showed that, the sequence was 202 nucleotides long. Blast analysis of C1 gene fragment of *psittacine Circovirus* in gene bank revealed 10 similar sequences in USA and Italy with their accession numbers (Fig 2). When nucleotide sequence of C1 gene of *Circovirus* were compared to other sequences in gene bank showed 95.5-100% identity and identical to other described *Circoviruses* elsewhere. Comparative nucleotide sequence analysis of red rumped parakeet strain with ten previously published strains was performed (Table 1). Compared with the consensus sequence showed two nucleotide exchanges at positions 16 and 126 but not effect on amino acid translation (Fig 2).

Amino acid sequence

C1 gene nucleotide sequence of *psittacine Circovirus* were translated into amino acids and compared with other 10 sequence in gene bank. No variation in deduced amino acids in Egyptian isolate compared to other 10 isolates elsewhere (Fig.3). All sequences are similar with 100% identity.

Phylogenetic tree

Phylogenetic tree constructed to C1 gene of *psittacine Circovirus* recovered from red rumped parakeet

showed that the Egyptian isolate sequence was clustered in clade closed to 3 sequences in USA (AY518900.1, AY521234.1 and AY521235.1) and separated from other 5 sequences (Fig. 4).

Amplification of T gene of Polyomavirus in psittacine birds using PCR

T gene of *Polyomavirus* was amplified in pooled feather and pooled tissue samples of three species of psittacine birds (parakeet, budgerigar and love birds). PCR results showed a predicted band at 310 bp when electrophoresed and separated in agarose gel (Fig.5). A total of eleven PCR positive feather sample out of 50 birds were positive to *Polyomavirus* with a total percentage of 22%. Meanwhile, a total of 10 PCR positive samples were recovered from pooled tissue samples with positivity percentage of 20.

Distribution of *Polyomavirus* infection in feather sample were higher than in the pooled tissue samples.

Sequence analysis of T gene of Psittacine polyomavirus

Nucleotide Sequence

Sequencing and analysis of PCR positive samples to *polyomavirus* from budgerigar our samples Egypt showed an amplified fragments of T gene of *polyomavirus* genome. Analysis of the nucleotide sequence revealed that the sequence was 297 nucleotides long. Blast analysis of T gene fragment of *psittacine polyomavirus* revealed 18 similar

sequences in gene bank with their accession numbers (Fig 6). When nucleotide sequence of T gene of *polyomavirus* was compared to other sequences in gene bank showed 99.3% to 100% identity. Only one nucleotide substitution was observed at position 203 and not effect on amino acid translation.

Amino acid sequence

Alignment of amino acid sequence of Egyptian *psittacine polyomavirus* with other sequences in gene bank showed 98 amino acids were translated, all translated amino acids were conserved across all the isolates examined. No variation observed at the level of amino acids and identical (100%) to all 17 sequence generated from gene bank (fig 7 and table2).

Phylogenetic tree

Phylogenetic tree constructed to T gene of *psittacine polyomavirus* recovered from Budgergie showed that, the Egyptian isolate sequence was clustered in clade closed to 4 sequences isolated from Germany, Japan, china and Poland but separated from other 13 sequences isolated from Newsland, Japan, China and Poland (Fig 8).

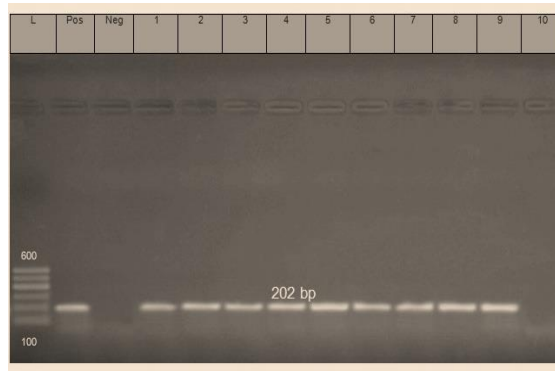


Fig. (1): Gel electrophoresis of C1 gene of *Circovirus* in *psittacine* birds

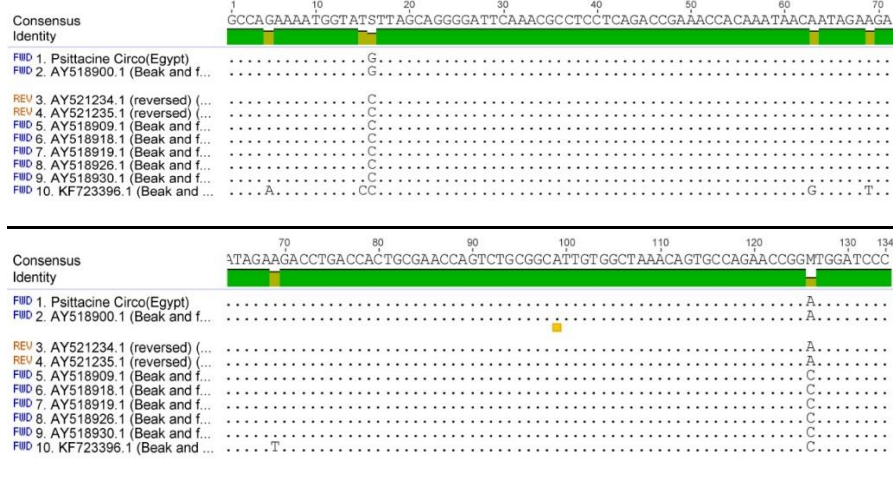


Fig. (2): Nucleotide alignment of C1 gene sequence of *psittacine circovirus* with 10 sequences generated from gene bank (n1=1-70, n2= 71-134)

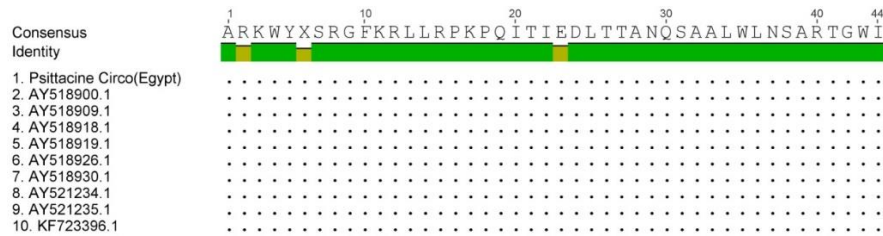


Fig. (3): Amino acid sequence alignment of Egyptian *psittacine circovirus* with other 10 sequences generated from gene bank.

Table (1): Nucleotide identity percentages of Egyptian Circovirus isolated from red rumped Parakeet compared to other 10 isolates in gene bank

Acc. No.	Circo(Egypt)	AY5189 00.1	AY5212 34.1	AY5212 35.1	AY5189 09.1	AY5189 18.1	AY5189 19.1	AY5189 26.1	AY5189 30.1	KF7233 96.1
Circo(Egypt)		100	99.3	99.3	98.5	98.5	98.5	98.5	98.5	95.5
AY5189 00.1	100		99.3	99.3	98.5	98.5	98.5	98.5	98.5	95.5
AY5212 34.1	99.3	99.3		100	99.3	99.3	99.3	99.3	99.3	96.3
AY5212 35.1	99.3	99.3	100		99.3	99.3	99.3	99.3	99.3	96.3
AY5189 09.1	98.5	98.5	99.3	99.3		100	100	100	100	97
AY5189 18.1	98.5	98.5	99.3	99.3	100		100	100	100	97
AY5189 19.1	98.5	98.5	99.3	99.3	100	100		100	100	97
AY5189 26.1	98.5	98.5	99.3	99.3	100	100	100		100	97
AY5189 30.1	98.5	98.5	99.3	99.3	100	100	100	100		97
KF7233 96.1	95.5	95.5	96.3	96.3	97	97	97	97	97	

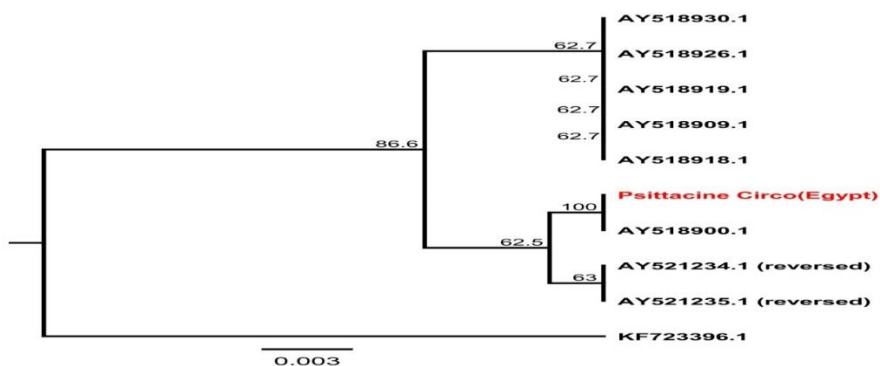


Fig.(4):Phylogenetic tree of C1 gene of Egyptian *Psittacine Circovirus* based on nucleotide sequence

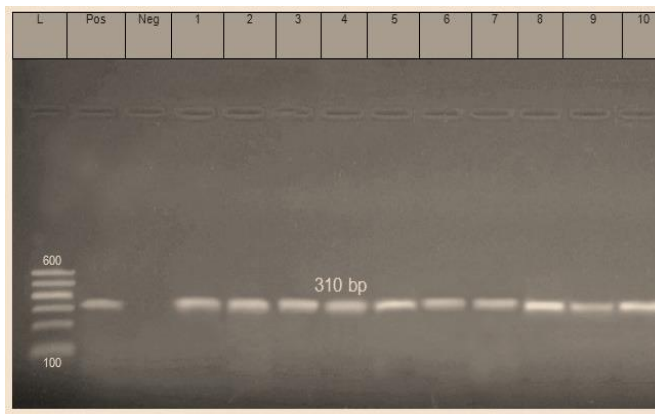


Fig. (5): Gel electrophoresis of T gene of *polyomavirus* in feather follicles of psittacine birds

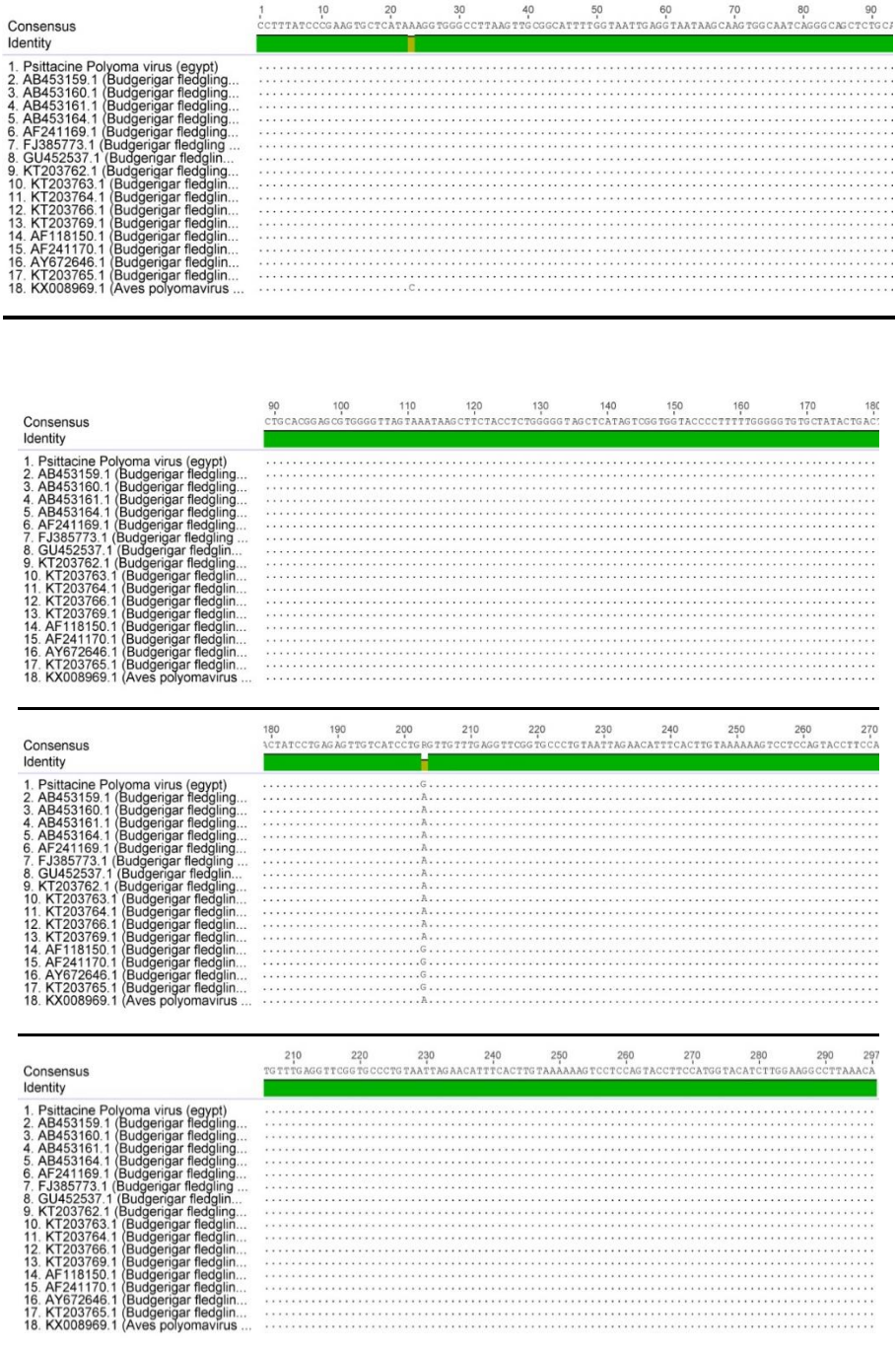


Fig. (6): Nucleotide alignment of T gene sequence of *psittacine Polyomavirus* with 18 sequences generated from gene bank (n1=1-90, n2= 91-180, n3=181-270, n4=211-297)

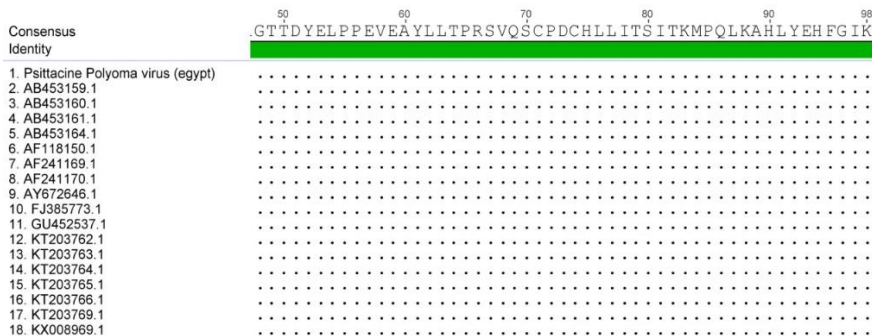


Fig.(7): Amino acid sequence alignment of Egyptian *psittacine Polyomavirus* with other 18 sequences generated from gene bank.

Table.(2): Nucleotide identity percentages of *Egyptian polyomaVirus* (T gene) isolated from Budgerigar and compared to 17 polyoma virus sequences in gene bank.

	Polyo m(eg ypt)	AB 453 159. 1	AB 453 160. 1	AB 453 161. 1	AB 453 164. 1	AF 241 169 .1	FJ3 857 73. 1	GU 452 203 537. 1	KT 203 762. 1	KT 203 763. 1	KT 203 764. 1	KT 203 766. 1	KT 203 769. 1	AF 118 150 .1	AF 241 170 646. 1	AY 672 646. 1	KT 203 765. 1	KX 008 969. 1
Polyoma (egypt)	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	100	100	100	100	99.3
AB453 159.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
AB453 160.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
AB453 161.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
AB453 164.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
AF241 169.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
FJ385 773.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
GU45 2537.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
KT20 3762.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
KT20 3763.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
KT20 3764.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
KT20 3766.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
KT20 3769.1	99.7	100	100	100	100	100	100	100	100	100	100	100	100	99.7	99.7	99.7	99.7	99.7
AF118 150.1	100	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	100	100	100	100	99.3
AF241 170.1	100	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	100	100	100	100	99.3
AY67 2646.1	100	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	100	100	100	100	99.3
KT20 3765.1	100	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	100	100	100	100	99.3
KX00 8969.1	99.3	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.3	99.3	99.3	99.3	99.3

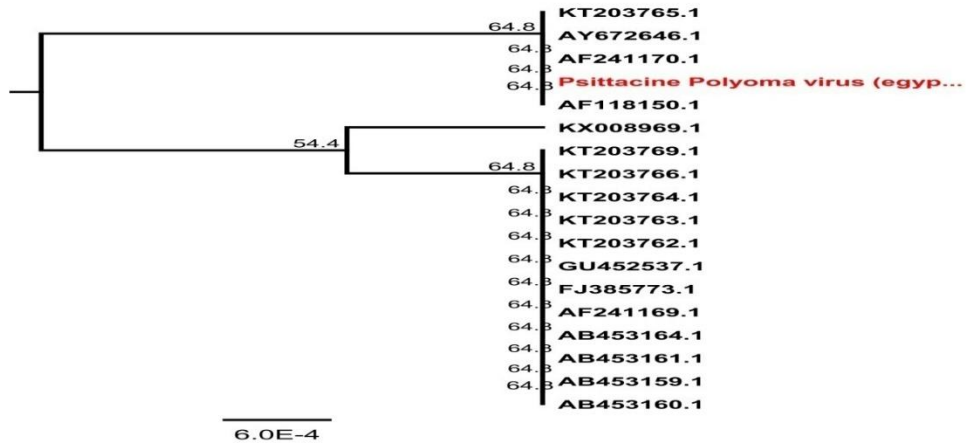


Fig.(8):Phylogenetic tree of T gene of Egyptian *Psittacine polyomavirus* based on nucleotide sequence

Discussion:

Infectious viral diseases of psittacine birds are the most common clinical problems in captive psittacine birds because of their association with acute death and difficulties in treatment and control. Among DNA viruses, *psittacine beak and feather disease virus* (PBFDV) and *avian polyomavirus* (APV) (*Bernier et al 1981 and Phalen et al 1993*) are the most important diseases affect a wide range of psittacine birds. In Egypt, information about virus infections in psittacine birds are limited and the objectives of this study were centered about DNA viruses in psittacine birds including PBFDV and APV in red rumped parakeet, budgerigar and love birds with respect to prevalence, clinical features, pathology, epidemiology and diagnosis of both viruses in birds of Egypt.

In this work, PCR was used to amplify C1 gene encoding capsid protein of Circovirus and T gene encoding of T antigen of avian polyomavirus in extracted DNA from psittacine birds

using a specific foreword and reverse primers. Total DNA extracted from 50 feather samples and pooled tissue samples were used as template for amplification. The predicted band size for these primers were a PCR product with a size of 202 bp for Circovirus and 302 bp for avian Polyomavirus respectively. The same results were obtained by (*Raue et al. 2004 & Piasecki and Wieliczko 2010*). PCR is a sensitive test and the method of choice for the diagnosis of PBFDV infections and avian polyoma infections. Several PCR protocols have been developed (*Ypelaar et al., 1999; Kiatipattanasakul-Banlunara et al., 2002; Ritchie et al., 2003*). With regard to PBFDV epidemiology, the C1 region coding the capsid proteins might be of particular interest as it should reflect virus host interactions as well as the selective pressure of the immune system. PCR positive results of Circovirus infection and avian Polyomavirus infection in different bird species of Egypt were recorded. Total positivity percentage of

Circovirus in feather sample (32%) is higher than pooled tissue samples (20%). PCR protocols are known to be labor-saving, time-saving and cost-saving. They also reduce the risk of laboratory contamination (*Raue et al. 2004*). Our PCR prevalence data are similar to those reported by *Bert et al., (2005)* but much lower compared to those reported by (*Rahaus, et al., 2003*) who found a much higher prevalence (39%) of BFDV DNA in feather samples collected from 146 clinically normal psittacine birds and even non psittacine birds in Germany.

In our study, the percent of nucleotide homology of the C1 coding region fragment of psittacine Circovirus compared to all published C1 sequences in GenBank ranged from 95.3 % to 100% (Table 1). Comparative nucleotide sequence analysis of red rumped parakeet strain of PBFDV with ten previously published strains recorded, showed two nucleotide exchanges at positions 16 and 126 but not effect on amino acid translation. Amino acid alignment of Egyptian psittacine Circo virus sequence indicated that the sequence of the C1 region was 100% identical to the other 10 published sequences in gene bank .Phylogenetic tree constructed to C1 gene of psittacine Circovirus recovered from red rumped parakeet bird showed that the Egyptian isolate sequence was clustered in clade closed to 3 sequences in USA (AY518900.1, AY521234.1 and AY521235.1) and separated from other 5 sequences elsewhere. These results supported by (*Ritchie et al. 1990*) who stated that, PBFDV is a genetically diverse virus and there

have broad genotype lineages aligned to the major families of psittacine birds namely the cockatoos, loriids and other parrots. BFDV isolates harvested from a diverse range of psittacine genera were found to be antigenically similar. Phylogenetic tree constructed in this study, based on nucleotide sequences of polyoma T antigen predicted in, showed that, Egyptian budgerigar strain was clustered in clade closed to 4 sequences isolated from Germany, Japan, china and Poland but separated from other 13 sequences isolated from Newland, Japan, China and Poland. *Avian Polyomavirus* in this study showed a broad diversity indicating an independent evolution of these proteins in *avian polyomaviruses* over a longer period of time, as demonstrated by separate branching in a phylogenetic tree (*Johne et al. 2007*). Alignment results and blast analysis of Egyptian *psittacine polyomavirus* suggested that, T gene of psittacine polyomavirus had a high degree of nucleotide sequence homology with the published APV sequences in GenBank ranging from 99.9% to 100% (Table 2). Some researchers have reported a very high degree of similarity revealed by the APV sequences.as reported previously (*Rott et al. 1988, Phalen et al. 1999 and Johne et al. 1998*). A phylogenetic analysis of the genome sequences of all *polyomaviruses* shows a separate branching of the *polyomaviruses* of birds, indicating a separate grouping and phylogenetic development of these viruses (*Johne et al. 2006*). Analysis of the genome sequences shows that the main

differences between mammalian and bird *polyomavirus* genome sequences are found in the large T antigen encoding region (Phalen *et al.* 1993). Finally, Egypt is a large axial and central country in the world for migratory birds and domestic poultry, and therefore infection of birds should be a matter of great importance. Here, in this study, we detected APV and PBFV infection in psittacine birds through PCR and made an accurate diagnosis of the affected budgerigars, red rumped parakeet and love birds. Sequencing of APV and PBFV isolates from Egypt will allow the evolutionary pathways of these viruses to be explored. The results of this study will be helpful for further research in the fields of epidemiology and molecular biology, which will have great significance in prevention and control of APV and PBFV infection

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Competing interests:

None of the authors have any competing interests to declare.

References:

Bernier, G.; Morin, M. and Morsolais G.(1981): A generalized inclusion body disease in the budgerigar (*Melopsittacus undulatus*) caused by a papovavirus-like agent. *Avian Dis* 1981;(25):1083-1092.

Bert, E.; Tomassone, L.; Peccati, C.; Navarrete, M.G. and Sola, S.C.(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, 52(2): 64-68

Cooper, J.E.; Lawton, M.P.C. and Greenwood, A.G.(1986): papillomas in psittacine birds. *vet Rec* (119);535-536

Gough, R.E.; Drury, S.E.; Culver, F.; Britton, P. and Cavanagh, D.(2006): Isolation of a coronavirus from a green-checked Amazon parrot (*Amazona viridigenalis* Cassin). *Avian Pathology*, (35), 122-126

Grund, C.H.; Werner, O.; Gelderblom, H.R.; Grimm, F. and Koesters, J. (2002): Avian paramyxovirus serotype 1 isolates from the spinal cord of parrots display a very low virulence. *Journal of Veterinary Medicine Series B*, (49) 445-451

Johne, R. and Muller, H. (1998): Avian polyomavirus in wild birds: Genome analysis of isolates from Falconiformes and Psittaciformes. *Arch. Virol.* (143), 1501±1512

Johne, R.; Wittig, D. Fernandez-Luco; U. Hoffle, and H. Müller. (2006): Characterization of two novel polyomaviruses of birds by using multiply primed rolling-circle amplification of their genomes. *J. Virol.* (80):3523–3531

Johne, R. and Muller, J. (2007): Polyomaviruses of Birds: Etiologic Agents of Inflammatory Diseases in a Tumor Virus Family. *Journal of Virology*, 81(21), 11554-11559.

Kiatipattanasakul-Banlunara W, Tantileartcharoen R, Katayama KI, Suzuki K, Lekdumrogsak T, Nakayama H, Doi K(2002): Psittacine beak and feather disease in three captive sulphur-crested cockatoos

- (*Cacatua galerita*) in Thailand. *J Vet Med Sci.* 2002;(64):527–529.
- Kistler, A.L.; Gancz, A.; Clubb, S.; Skewes-Cox, P., Fischer K. and Sorber, K. (2008):** Recovery of divergent avian bornaviruses from cases of proventricular dilatation disease: identification of a candidate etiologic agent. *Virology Journal*, (5), 88
- Khalesi, B.; Bonne, N.; Stewart, M.; Sharp, M. and Raidal S. (2005):** A comparison of haemagglutination, haemagglutination inhibition and PCR for the detection of psittacine beak and feather disease virus infection and a comparison of isolates obtained from lorriids. *J Gen Virol* (86):3039–3046.
- McDonald, S. E.; Lowenstine, L. J. and Ardans, A. A. (1981):** Avian pox in blue-fronted Amazon parrots. *J. Am. Vet. Med. Assoc.*, 179(11):1218-22.
- Pass D.A. and Perry R.A. (1984):** The pathology of psittacine beak and feather disease. *Aust. Vet. J.* 1984;(61):69–74
- Phalen, D.N. and Wilson, V.G. Graham (1993):** American journal veterinary research, 54(12):2040-2047
- Phalen, D.N. (1998).** Avian polyomavirus. *Journal of the American Federation of Aviculture*, 25(5), 28-39
- Piasecki, T. and Wieliczko, A. (2010):** Detection of beak and feather disease virus and avian polyomavirus DNA in psittacine birds in Poland. *B Vet I Pulawy* 54:141–146
- Pillai, S. P.; Suarez, D. L.; Pantin-Jackwood, M. and Lee, C. W. (2008):** Pathogenicity and transmission studies of H5N2 parrot avian influenza virus of Mexican lineage in different poultry species. *Vet. Microbiol.* 129: 48–57
- Rahaus, M. and Wolff, M.H. (2003):** Psittacine beak and feather disease: a first survey of the distribution of beak and feather disease virus inside the population of captive psittacine birds in Germany. *J Vet Med B Infect Dis Vet Public Health.* 2003 Oct;50(8):368-71
- Raue, R.; Johne, R.; Crosta, L.; Bürkle, M.; Gerlach, H. and Müller, H. (2004):** Nucleotide sequence analysis of a C1 gene fragment of psittacine beak and feather disease virus amplified by real-time polymerase chain reaction indicates a possible existence of genotypes. *Avian Pathol* (33): 41- 50
- Raue, R.; V. Schmidt; M. Freick; B. Reinhardt; R. Johne; L. Kamphausen; E. F. Kaleta; H. Müller, and M. E. Krautwald-Junghanns. 2005.:** A disease complex associated with pigeon circovirus infection, young pigeon disease syndrome. *Avian Pathol.* (34):418–425
- Ritchie, B.W.; Niagro, F.D.; Latimer, K.S.; Lukert, P.D.; Steffens, W.L.; Rakich, P.M. and Pritchard, N. (1990):** Ultrastructural, protein composition, and antigenic comparison of psittacine beak and feather disease virus purified from four genera of psittacine birds. *Journal of Wildlife Diseases*, (26):196–303.
- Ritchie, P. A.; Anderson, I. L. and Lambert, D. M. (2003):** Evidence for specificity of psittacine beak and feather disease viruses among avian hosts. *Virology* (306), 109-115
- Rott, O.; Kro\$ ger, M.; Mu\$ ller, H. and Hobom, G. (1988):** The genome of budgerigar -edgling disease virus, an avian polyomavirus. *Virology* (165), 74±86.
- Simpson, C.F.; Hanley, J.E. and Gaskin, J.M. (1975):** Psittacine herpesvirus infection resembling Pacheco's parrot disease. *Journal of Infectious Diseases*, (131), 390-39
- Standley, DM.; (2013):** MAFFT multiple sequence alignment software version 7: improvements in performance and

usability .Mol Biol Evol. 2013 Apr;30(4):772-80

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5(2011): molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. (28):2731–2739

Todd, D. (2004): Avian circovirus diseases: lessons for the study of PMWS. Veterinary Microbiology, (98), 169–174

Van den Brand; J. R. Manvell; P. Guntram; MJ Kik and G.M .Dorrestein. (2007): Reovirus infections associated with high mortality in psittaciformes in The Netherlands. Avian Pathol (36), 293-299.

Ypelaar, I.; Bassami, M.R.; Wilcox, G.E.and Raidal, S.R.(1999): A universal polymerase chain reaction for the detection of psittacine beak and feather disease virus. Veterinary Microbiology(68), 141-148.

الملخص العربي

نظراً لأهميه طيور الزينه فى الاستمتاع برويتها وإستئناسها وإستخدامها للزينه وتربيتها فى المنازل فقد أخذت أهتماما كبيرا خصوصا فيما يخص مسببات الامراض التى تصيبها وخاصة الأمراض الفيروسيه ومن أهم طيور الزينه التى تربي فى المنازل هى طيور فصيله البيغاوات .تم تجميع عدد إجمالى 50 طائر من طيور فصيله البيغاوات.تم استخدام انزيم البلمره المتسلسل التقليدي أثبتت النتائج أن أعلى نسبة أنتشار لفيروس السركو كانت فى طيوربيغاء الظهر الأحمر يليه طائر الدره ثم طيور الحب بنسبه 41.66% & 26.92% & 33.33% على التوالي أما عن تواجد فيروس السركو فى الطيور صغيره العمر فكانت 42.85% & 26.66% & 37.5% فى بيغاء الظهر الأحمر وطيور الدره وطيور الحب على التوالي وهى أعلى من نسبتها فى الطيور البالغه أما عن نسبة تواجد فيروس السركو فى الأعضاء الداخليه فهى 28.57% & 20% & 12.5% على التوالي .وقد أثبت أيضا أصابه الطيور بفيروس البوليوما فى الريش فى عدد 11 عينه من إجمالى 50 بنسبه إجماليه 22% أكثر من أصابه الأعضاء الداخليه بنسبه 20%. أما عن إنتشار الفيروس فى أنواع الطيور المختلفه كانت نسبتها 23% & 23.7% & 16% فى طيور بيغاء الظهر الاحمر وطائر الدره وطيور الحب على التوالي وكان أعلى إنتشار للفيروس فى طائر الدره الاستراليه يليه بيغاء الظهر الأحمر ثم طيور الحب وقد أثبت تحليل البلاست أن تتابعات الجين سى 1 لفيروس السركو المتواجد بمصر متشابهه تماما مع 10 تتابعات أخرى لفيروسات معزوله من أمريكا وإيطاليا. وبمقارنه هذه التتابعات مع العشره المتشابهين معها فى بنك الجينات وجد هناك تطابق بينهما من 95.5% إلى 100% مع وجود تبادل لبعض القواعد عند الموقع 16 و 126 ولكن هذا التبادل لا يؤثرعلى تتابعات الأحماض الأمينيه المترجمه. أوضح تحليل البلاست للجين تى الخاص بفيروس البوليوما المعزول من طائر الدره الاستراليه المتواجده بمصر أن هناك عدد 18 تتابع لنفس الجين مسجلين على بنك الجينات لفيروسات معزوله من بعض الدول الأجنبيه وعند مقارنه تتابع الفيروس المعزول مع التتابعات الأجنبيه وجد هناك تطابق للقواعد بينهما يتراوح من 99.9% إلى 100% مع وجود بعض التبادل لبعض القواعد عند الموقع 203 فقط لكنها لاتؤثر على الأحماض الأمينيه المترجمه