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Detection of Gammapolyomavirus Avis in New Species of Commercial and Conservation Captive Psittacines in Brazil

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Simple Summary: Adult native and young exotic psittacines at human care exhibiting unspecific clinical signs were detected APyV positive. However, none of the observed lesions could be linked to the presence of APyV. The eventual lack of correlation between clinical signs and lesions and the presence of APyV poses as an obstacle for health management in captivity. Considering that the captivity of psittacines, for commercial of conservation purposes, enables transmission, the laboratory monitoring is considered essential for the implementation of biosecurity and preventive strategies.

Abstract: The captive native adult psittacines at human care, *Amazona aestiva* (blue-fronted parrot) and *Ara chloropterus* (red-and-green macaw), clinically affected with reduced activity/appetite and poor feathering, one found dead *Anodorhynchus hyacinthinus* (hyacinth macaw) and two exotic dead-in-nest *Psittacula krameri* (rose-ringed parakeet) were examined for signs and for ante- and post-mortem lesions, and for the presence of avian polyomavirus (APyV; *Gammapolyomavirus avis*) T/t genes. All individuals were tested-positive for APyV in skin and/or in other tissues such as blood, brain, liver, skin and spleen, and all were tested-negative for psittacine circovirus (BFDV). However, no association was found between the detection of APyV and the presence of macro or microscopic lesions. The APyV sequences detected in the *A. aestiva* and *P. krameri* were grouped together with high identity, which may suggest common origins. However, the sequence detected in *A. hyacinthinus* was dissimilar and grouped separate. The sequences detected had high identity with T/t gene sequences of strains previously described in distinct continents. The evaluation of avian species for APyV may not be available nor demanded in the international avian trade and may represent an avian health hazard. The biosecurity of the commercial avian pet flocks and the captive avifauna in rehabilitation is considered essential.

Keywords: avian virus infection; psittaciformes; *Polyomaviridae*; captive psittacines; feather impairment; clinically affected birds

1. Introduction

The budgerigar (*Melopsittacus undulatus*) fledgling disease virus was the first avian polyomavirus (APyV) described [1]. APyV causes a disease in young psittacines characterized by ascites, hepatitis, and crop stasis, described with high mortality in nestlings, but with inapparent infection in adults, except for mild feather dystrophy [2]. The prevalence of APyV infection in psittacines may be unknown and varied in most regions and has been reported sporadically [3,4] and non-psittacine occurrences have been documented in buzzards (*Buteo buteo*) and falcon (*Falco tinnunculus*) in Germany [5], Adélie penguin (*Pygoscelis adeliae*) in Ross Island, Antarctica [6], green aracaris (*Pteroglossus viridis*), laughing kookaburra, Lady Ross turaco and zebra finches [7]. The PCR

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detection of APyV in blood is considered a sanitary risk as the individual must considered to be shedding the virus [8]. In humans, polyomaviruses antibodies are found in >90% of the population and were described as causing lifelong infections with latency in the urinary epithelium and cyclic shedding into the urine [9], although this characteristic was not demonstrated for avian strains.

Human Merkel cell polyomavirus MCPyV is associated with the development of Merkel cell carcinoma (MCC) and is considered a group 2A carcinogen (probably carcinogenic to humans) by the International Agency for Research on Cancer [10]. A Sertoli cell tumor in a Gouldian finch was described as associated to polyomavirus infection [11] and an APyV was described as capable of transforming BHK-21 cells, similarly to the mammalian polyomaviruses [12].

APyV forms a distinct group within *Polyomaviridae* [13,14]. APyV of a chicken (BFDV-2) and a parrot (BFDV-3) were determined as closely related to a budgerigar virus (BFDV-1) and proposed to integrate a new subgenus *Avipolyomavirus* [1]. The most recent nomenclature describes APyV as *Gammapolyomavirus avis* species (14 ICTV, *Polyomaviridae*, 2024) and the acronym "BFDV" is considered confusing with that of beak and feather disease virus, an avian circovirus (*Circoviridae, Circovirus parrot*; 15 ICTV, *Circoviridae*, 2024).

This report describes the detection of APyV in psittacines in Minas Gerais, Brazil, asymptomatic or clinically affected by unspecified morbid conditions, characterized by reduced activity and appetite, emaciation and poor feathering of adults, or mortality in the very young. The potential implications of the infection to the biosecurity of raising and commercializing exotic avian pet species at proximity to triage/rehabilitation native avifauna are considered.

2. Materials and Methods

2.1. Psittacines

The specimens included in this study (Table 1) were collected from individuals with unspecific signs of reduced activity and appetite, and poor feathering. Psittacines that died or were euthanized due to suffering and/or an eventual sanitary risk to others were submitted to complete necropsy. One *Amazona aestiva* was kept under human care at a triage conservation facility. The *Psittacula krameri* (n=2) were raised at a commercial establishment. Samples collected at the necropsy for histopathology and/or molecular diagnosis included blood, CNS, feather, liver, muscle, skin and spleen. Blood and feather/skin samples were also obtained from *Ara chloropterus* (n=4) and *Anodorhynchus hyacinthinus* (n=1) that were kept under human care at the BH Zoo (Zoological Garden, Belo Horizonte, Brazil).

Table 1. Local avian polyomavirus partial large T/t gene sequences positive individuals.

Host species	Sample	Origin, State, Country	APyV Accession Number	*Aspergillus fumigatus*	*Chlamydia psittaci*	*Circovirus parrot*	*Mycoplasma* spp.
Anodorhynchus hyacinthinus (n=1)	Blood	Zoological Garden, Minas Gerais, Brazil	PP213160	NEG	NEG	NEG	NEG
Ara chloropterus (n=4)	Blood	Zoological Garden, Minas Gerais, Brazil	PP213161	NEG	NEG	NEG	NEG
Ara chloropterus (n=4)	Blood	Zoological Garden, Minas Gerais, Brazil	PP213162	NEG	NEG	NEG	NEG
Psittacula krameri (n=2)	Central nervous system	Commercial grower, Minas Gerais, Brazil	PP213163	NT	NT	NEG	NT

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Amazona aestiva (n=1)	Blood-liver-skin-spleen-	Conservation facility, Minas Gerais, Brazil	PP213164	NEG	NEG	NEG	NEG

NT: Not tested.

2.2. Post-Mortem Examination and Histopathology

Necropsies were performed in four *A. chloropterus*, one *A. hyacinthinus*, one *A. aestiva*, and two nestling *P. krameri*. The four *A. chloropterus* were euthanized due to a previous molecular diagnosis of avian polyomavirus infection (detailed below) as an infection control strategy. The *A. aestiva* and the two nestling *P. krameri* died with the progression of an unknown disease and samples of the liver, lung, brain, kidney, testis/ovary, heart, gizzard, proventriculus, esophagus, crop, small and large intestine, pancreas, skin, spleen, adrenal, sciatic nerve and skeletal muscle were fixed in 10% buffered formalin for 48 hours, embedded in paraffin, sectioned in a microtome (3-4µm-thick sections), stained with hematoxylin and eosin (H&E), and evaluated under light microscopy [16].

2.3. Molecular Techniques and Phylogeny

DNA was extracted from tissue or blood samples as previously described [17], and used as template for a PCR reaction to partially amplify the large T/small t antigen genes of *Gammapolyomavirus avis*, with primer pairs 5'CAAGCATATGTCCCTTTATCCC-3' and 5'-CTGTTTAAGGCCTTCCAAGATG-3' with an expected product of 310 bp [5]. Psittacine beak and feather disease virus (BFDV, *Circovirus parrot*) was investigated using the previously described primers 5-AACCCTACAGACGGCGAG-3 and 5-GTCACAGTCCTCCTTGTACC-3 [18], with an expected product of 717 bp, designed for amplifying part of the VP1 gene region of open reading frame 1 (ORF-1). *Aspergillus fumigatus* (AfLC2s 5'- AATGCACGATACTGTAGGATCTG -3 ' and AfLC2as 5 '-TGCATTGGATTAGCCATAACA -3') [19], *Chlamydia psittaci* Cp-F-5′-ACTACGGAGATTATGTTTTCGATCGTGT-3′ and Cp-R-3′TCTTGGAGCGTYGGTGCACG-5′ [20], *Mycoplasma* sp. (oligonucleotides F5′-ACACCATGGGAGYTGGTAAT-3′ and R3′-CTTCWTCGACTTYCAGACCCAAGGCAT-5′) [21], with a 370-500 bp product, for amplifying part of the 16S ribosomal RNA gene), were also investigated using previously described primers The molecular mass of the PCR products was estimated by comparing with a 100 bp ladder (Promega, Fitchburg, WI, USA). The PCR products were sequenced [22] and sequences were aligned (ClustalW) and the consensus determined for each host species. The phylogenetic relationships were determined to published sequences available online (GenBank, NCBI) and inferred using the UPGMA method (unweighted pair group method with arithmetic mean) of analysis [23] in MEGA XI [24].

The following APyV accession numbers of the sequences used for comparisons were obtained from the GenBank: NC_004800.1 Goose hemorrhagic polyomavirus in Germany; ON186484.1 and ON186483.1 of Bangladesh; OL348190.1, OL348191.1, OL348192.1 and OL348193.1 of *Agapornis fischeri* in Iran; AF241168.1 and AF241170.1 of *Melopsittacus undulatus* in Germany; NC_039052.1 of *Erythrura gouldiae* (Gouldian finch; Estrildidae, Passeriformes) in Poland; AF054397.1 of *Aratinga acuticaudata*, AF054392.1 of *Aratinga solstitialis*, AF054390.1 of *Agapornis personata* and AF054389.1 of *Eclectus roratus* in the USA. Human polyomavirus (LC416388.1 and MG241574.1) sequences were added as outgroup. The local isolates accession numbers are available in Table 1.

3. Results

All tested individuals were positive for APyV (Table 1). The Amazona aestiva, Ara chloropterus and Psittacula krameri individuals had reduced activity, loss of appetite and poor feathering, and the A. hyacinthinus individual died in the premises without previous clinical signs. However, none of the signs or lesions could be associated to the presence of APyV, as no typical histopathological lesions indicative of APyV were found. All the tested individuals were negative to BFDV (Circovirus

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parrot), Aspergillus fumigatus, Chlamydia psittaci (except P. krameri, not tested) and Mycoplasma spp. by PCR (Table 1).

The obtained sequences of APyV large T/small t antigen genes were phylogenetically characterized and the partial identity of the sequences found in A. aestiva (accession number PP213164) and P. krameri (PP213163) was demonstrated. (Figure 1), according to the UPGMA phylogenetic tree. The sequence detected in Anodorhynchus hyacinthinus (PP213160) had identity with strains described in Agapornis pullarius (ON186483.1) and Melopsittacus undulatus (ON186484.1). The sequence detected in the Ara chloropterus (PP213161) was grouped with a strain detected in Psittacula eupatria.

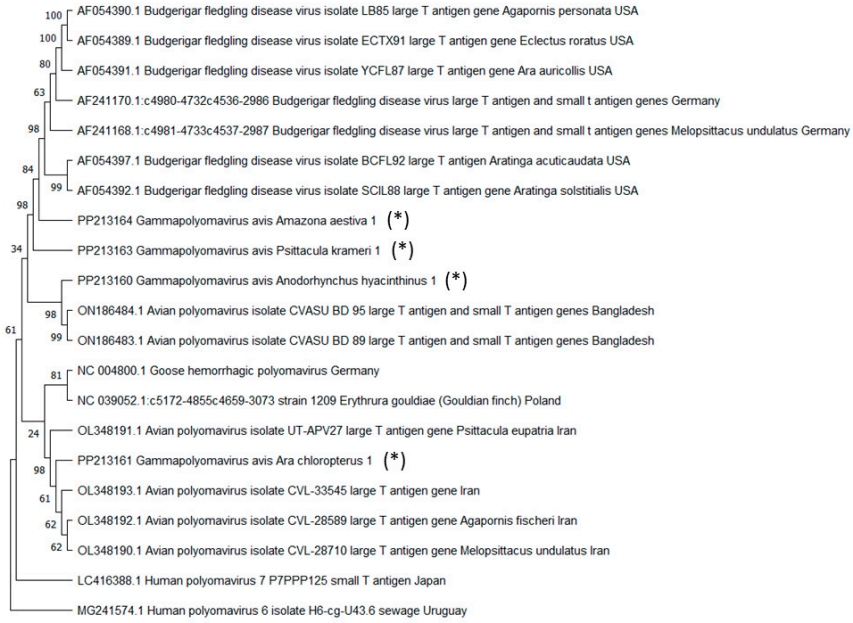

Figure 1. Evolutionary relationships of *Gammapolyomavirus avis.*

The evolutionary relationships were inferred using the UPGMA (unweighted pair group method with arithmetic mean) method of analysis. The PCR products were obtained using primers flanking the APV T/t-antigen gene, forward CAAGCATATGTCCCTTTATCCC and reverse CTGTTTAAGGCCTTCCAAGATG (Johne & Muller, 1998). The local sequences are highlighted (*). The sequence detected in *Anodorhynchus hyacinthinus* grouped in 98% of trees with strains described in Bangladesh in *Agapornis pullarius* (red-headed lovebird) (ON186483.1) and budgerigar (ON186484.1. The sequence detected in *Ara chloropterus* was grouped with high identity (98%) with a strain detected in *Psittacula eupatria* in Iran (OL348193.1). The sequences detected in *A. aestiva* and *Psittacula krameri* were grouped closely together in 98% of trees, and the *A. aestiva* sequence branched in 84% of trees with sequence (AF241168.1) described in *Aratinga solstitialis* and *Aratinga acuticaudata* of the USA. Considering the identity of the large T/t gene sequences among different host species, the potential interspecies transmission of avian polyomaviruses has a probability of occurring, or may have a common ancestral origin. Human polyomavirus T/t protein gene sequences (LC416388.1 and MG241574.1) were used as external taxon sequences. The evolutionary history was inferred using the UPGMA method [23]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 21 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1170 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [24].

4. Discussion

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4.1. Psittacines

The young individuals included in this study were 10-day old P. krameri nestlings (n=2) from a commercial pet grower, which also maintained other commercial exotic species. Except for APyP, no other pathogen was detected in these and considering the history of antibiotic medication, associated with the histopathological lesions, might indicate a case of toxicity. It has become common to commercial growers to purchase and administer medication which, eventually, may become toxic [25]. The histopathology is described in the next section (4.2).

The A. chloropterus individuals had clinical signs during the course of one year, with progressive weight loss, plucking or damaged feathers, constant reinfections by endo and ectoparasites, diarrhea, and apathy. The adult A. aestiva and the nestlings P. krameri died with the course of an unknown disease. The Anodorhynchus hyacinthinus, was found dead in the housing premises, without precursor signs, and was more than 30 years old. Macaws may be naturally infected as young and develop clinical signs and mortality from 4 to 14 weeks of age [8], and subsequently remain virus positive in blood and cloaca, shedding virus in droppings, as shown her for Ara ararauna. The detection of APyV in the adult pittacines, as described here, after a long-term in captivity, suggests that the opportunity of transmission to cohabitants may have occurred. Avian polyomavirus was described to induce lifelong infection and the concurrent infection with the psittacine circovirus of the beak and feather disease may be determinant of the clinical-pathological expression of APyV [8]. However, the absence of Parrot circovirus in our specimens might have resulted in a lesser impact of APyV.

Although budgerigars are severely affected as nestlings, adults may be clinically normal in all aspects, including fertility and reproduction, and virus may be found in most organs [26]. In nestling budgerigars, the fatality rate is close to 100% and the surviving young develop a persistent feathering disorder [27]. However, despite of the higher clinical importance in young birds, APyV infection in adults may result in severe kidney lesions characterized by membranous glomerulopathy [28].

Previously described histopathologic features of lesions in clinically affected were typically found in young birds, and revealed the proliferations of small basophilic tumor cells in the dermis and hypodermis of humans with Merkel cell carcinoma [29], and were not found in the currently examined birds. However, a better characterization of the infection may require determining a relationship between the presence of the virus and the observed lesions, such as using immunohistochemistry [30,31], prefferably using monoclonal antibodies to conserved antigens of APyV, such as used for the detection of Merkel cell carcinoma polyomavirus [32]. Another future strategy might employ fluorescence in situ hybridization (FISH) with oligonucleitides complementary to APyV conserved sequences, such as the 60 nt universal probes used to detect dengue virus of four different serotypes [30] .

The risk of APyV may arise with the proximity to avian flocks of unknown health status, different categories and taxonomic groups. These risk conditions are present for all the specimens here studied. For instance, non-psittacine avian species may be infected by avian polyomavirus and may have a role as souces. Chickens were shown to be infected by a APyV, although chicken strains are considere different from psittacines strains [33]. APyV antibodies were detected in cockatoos, cockatiels, laughing kookaburra, Lady Ross turaco, zebra finches and lesions in green araçaris (Pteroglossus viridis), included generalized hemorrhage, splenic necrosis with clear to slightly basophilic nuclear inclusion bodies in splenocytes with karyomegaly [7]. Additional thirty polyomaviruses were identified up to 2015 from mammalian species, with species-specific polyomaviruses detected in apes, bats, cows, hamsters, horses, humans, mice, monkeys, rabbits, raccoons, sea lions, and a variety of avian species [34].

4.2. Post-Mortem Examination and Histopathology

The only young individuals included in this study were 10 day-old P. krameri nestlings, original from a commercial grower, which also maintained other commercial exotic species. The clinical history of the nestlings at 8-14 days post-hatch indicated subcutaneous hemorrhage at the pelvic limbs and sudden death. Medication with antibiotics (unknown) during the first two days post hatch

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was a usual procedure. At the necropsy, the 9-10 cm long nestlings (from the occipital to the synsacrum) had normal muscular development, large (approximately 2.0 x 3.5 cm) areas of subcutaneous hemorrhage on the abdominal region and at the extension of the pelvic limbs. All had enlarged liver, with rounded edges, yellowish and friable, and the ingluvius was distended with pasty feed. All had moderately enlarged spleen with multifocal white spots and intestinal lumen with watery content. Microscopically, all P. krameri (2/2) had livers with multifocal coalescing central-lobular lympho-granulocytic infiltrations, with coagulative necrosis and disorganized highly eosinophilic hepatocytes, a few with pycnotic or absent nuclei. Lesions could be associated to the history of given medication with unknown antibiotics. At the periportal areas, the hepatocytes were normal. However, in our study, no association was found among the described lesions and the presence of APyV.

The macro and microscopic lesions found in the A. chloropterus individuals were also nonspecific, with emaciation (2/4) with muscular hypotrophy (2/4), focal area of apteria (1/4), dermatitis (3/4), brain lipofuscinosis (4/4), interstitial nephritis (2/4), pigmentary nephrosis (2/4), renal intratubular mineralization (2/4), renal amyloidosis (2/4), hepatic steatosis (2/4), hepatic portal fibrosis and bile duct hyperplasia (3/4), granulomatous hepatitis (1/4), necrotizing and granulomatous splenitis (1/4), pulmonary hemorrhage and congestion (3/4), lymphoplasmacytic bronchitis (2/4), anthracosis (4/4), lymphocytic orchitis (1/4), testicular degeneration (1/4), lymphoplasmacytic enteritis (1/4), and intestinal nematodes (2/4). The A. hyacinthinus had a hepatocellular carcinoma with metastasis to the lungs, which was the cause of death. Therefore, in the evaluated group of birds the diagnosis of avian polyomavirus presence was based on molecular tools, since none of the A. chloropterus nor the A. hyacinthinus had lesions attributable to APyV infection [35]. In this study, the A. hyacinthinus individual was considered an asymptomatic host and the clinically affected psittacines did not express signs or lesions typical of APyV. Although the non-specific clinical signs observed in the A. chloropterus may be considered compatible with avian polyomavirus infection, the microscopic lesions did not support this assumption and APyV presence was interpreted as incidental. Other findings in the A. chloropterus included pigmentary nephrosis with intratubular mineralization, testicular degeneration with interstitial cell hyperplasia, myocardial fibrosis, intestinal amyloidosis and necrosis and an ulcerative and heterophilic dermatitis.

A DNA in situ hybridization method was employed to detect virus DNA in paraffin-embedded tissue sections of naturally or experimentally infected species, with the detection of APyV and avian adenovirus in nuclear inclusions and psittacine beak and feather disease virus and Pacheco's parrot disease virus in cells with or without inclusions [36].

4.3. Molecular Techniques and Phylogeny

The consensus sequences of the APyV T/t proteins encoding genes were determined separate for each psittacine species investigated. The partial identity of the sequences to previously published sequences of different continents suggests that the international trade and the interspecies transmission may have a role in the epidemiology. The A. aestiva (accession number PP213164) and P. krameri (PP213163) polyomavirus isolates sequences were grouped together in 84% of trees (Figure 1), according to the UPGMA phylogenetic tree, suggesting a potential role for the local commerce of exotic companion species in the dissemination. Indeed, the A. aestiva sequence branched in 98% of trees with a sequence (AF241168.1) described in a budgerigar in Germany. Also, the sequence detected in Anodorhynchus hyacinthinus (PP213160) was grouped in 98% of trees with identity with strains described in Bangladesh, in Agapornis pullarius (ON186483.1) (red-headed lovebird) and budgerigar (ON186484.1). The sequence detected in Ara chloropterus (PP213161) was grouped, also with high identity (98%), with a strain detected in Psittacula eupatria in Iran (OL348193.1). All these detection and sequence comparisons exemplify the potential epidemiological association with the avian commerce.

The genomes of twenty variants of APyV of different hosts, different geographical regions and time of collection were compared and suggested that species-specific pathotypes did not appear to

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have evolved [8]. The comparison of strains obtained from different hosts resulted in similarity which suggested a single serotype and possibly single genotype [5]. APyVs were isolated from psittacine of four species and their whole genome sequences were genetically analyzed, indicating a single amino acid code substitution (nonsynonymous), with little effect on virus propagation [37].

The A. aestiva and P. krameri sequences identity could be related a spillover and the interspecies infection was considered a possibility. For instance, the previously described sequence (NC039052.1) found in a Gouldian finch in Poland, was grouped with a sequence found in goose hemorrhagic disease in Germany (NC004800.1). Polyomavirus was detected from Adélie penguin (Pygoscelis adeliae) fecal matter in Ross Island, Antarctica [Adélie penguin polyomavirus (AdPyV)], which shared about 60% pairwise identity with all APyV [6].

The large T protein is essential for replication by activating quiescent cells and in transformation, by interfering with cell division regulating mechanisms, and the small t protein promotes viral genome replication through the activation of cell phases G1 and S [38]. These genes have been used as targets for the molecular diagnosis and phylogeny [5]. The T-antigen gene sequences may vary among the mammalian polyomavirus species, although the overall arrangement of major functional protein domains is broadly conserved [39], possibly enabling the interspecies infection in mammals, including humans such as from bovines [40] and chimpanzees [41].

4.4. Epidemiology

In our study, exotic and native psittacine species were detected positive for APyV. The results suggest the possibility for a local risk, as APyV was detected in clinically normal individuals which could be source of infection, as considered previously [42]. Psittacula krameri is a very popular exotic species and its commercial breeding is growing in Brazil [43], due to its intelligence and being highly appreciated as companion bird. However, health standards are needed for the safe commerce of pet birds and the commercial breeding facilities may benefit from raising healthier flocks. Healthier breeders will produce healthier progenies, with higher productivity and lower losses. The health recommendations may include APyV and other signifcant pathogens, such as avian circovirus, Chlamydia psittaci and Macrorhabdus ornithogaster, all routinely detected in pet birds in our laboratory.

The literature description of the complete genome sequence of APyV detected in the liver of a Gouldian finch enabled the demonstration of highest homology (71%) to a crow polyomavirus (accession number DQ192570) [44]. These findings suggest interspecies transmissions. Indeed, a potential interspecies transmission of avian polyomaviruses may have locally occurred, as the sequences detected in the A. aestiva and P. krameri had high identity (Figure 1). Other APyV of two distant host taxonomies were grouped in the tree, such as a goose polyomavirus NC_004800.1 (unspecified, Anatidae, Anseriformes), described in a goose with hemorrhagic polyomavirus infection in Germany, was grouped with a sequence of Gouldian finch from Poland, in 81% of the trees.

APyV was described associated to severe disease in young psittacines [1], with high mortality of nestlings, but with inapparent infection in adults [2]. In most regions, the prevalence of APyV has been reported sporadically [3; 4]. APyV threat to conservation may be underestimated and a continuing challenge, due to the lack of regular testing and lack of a validated vaccine and vaccination protocol. However, an oil-emulsioned inactivated vaccine was given subcutaneously to Ara ararauna chicks, and although causing granulomata at the site of injection, resulted in higher resistance to challenge, as revealed by lower measurable neutralizing antibody post-challenge, as compared to naïve chicks [45]. Safer and more effective vaccines are needed for promoting the protection of valuable flocks for commercial and conservation purposes [46].

Natural antibodies to APyV were detected in both wild and captive psittacine species in Australia. Ninety-six (64.4%) wild sulphur-crested cockatoos (Cacatua galerita) (n=144) and two of 17 wild long-billed corellas (Cacatua tenuirostris) of three regions in New South Wales (NSW, Australia), but no wild galahs (Eolophus roseicapillus) (n=107) had positive (≥ 1:32) neutralizing antibody titres to AvPyV [47]. APyV, in addition to being known to cause infection and mortality in

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a diversity of psittacine species, was detected in buzzards (Buteo buteo) and in a falcon (Falco tinnunculus) in Germany, and in wild-caught (Mozambique) lovebirds (Agapornis pullaria), considered fatal, and caused by strains very closed related to other APyV [5]. Non-budgerigar psittacines were tested for APyV viremia, virus shedding and specific antibodies, revealing 4.5 month long cloacal shedding in nestlings [48]. New Zealand's endemic avian species are threatened by APyV, beak and feather disease virus (BFDV), and avian malaria, with APyV (22%) and Plasmodium relictum (30%), detected in introduced captive budgerigars in exhibitions, although BFDV (circovirus) was not detected in these specimens [49].

In a study elsewhere, clinically healthy breeding budgerigars of an aviary positive for APyV DNA were examined and shown to harbor APyV in most organs and systems, although at lower concentrations in breeding than in nonbreeding birds [29]. APyV infected individuals could be subclinical sources of infection, as 2.7% APyV positives were clinically normal [44]. APyV was described along with the psittacine beak and feather disease virus (PBFDV) in 3.3% of the native psittacine wildlife of Costa Rica, affected by feather impairment and other clinical signs, with a higher risk for psittacines living in shelters and rescue centers as compared to veterinary clinics [50].

Considering occurrence, in São Paulo (Brazil), 17% of psittacines were tested positive for APyV DNA, including Agapornis personata, Amazona aestiva, Eclectus roratus, Melopsittacus undulatus, Psittacus Erithacus, and Triclaria malachitacea [51]. A captive psittacine population of eighteen breeding and four commercial centers was tested (PCR) in Italy, revealing an occurrence of 0.79% positivity, suggesting a higher health standard relative to APyV (52). In New Zealand, the occurrence of APyV was evaluated in order to monitor and mitigate its impact in threatened captive native species, in addition to other pathogens, revealing an occurrence of 22% in Melopsittacus undulatus, with VP1 gene sequence divergence by 32% to previously reported strains [50].

5. Conclusions

Our preliminary findings indicate the occurrence of APyV in the captive psittacine species Amazona aestiva, Anodorhynchus hyacinthinus, Ara chloropterus and Psittacula krameri individuals, affected with unspecific clinical signs and lesions. The detection may reveal the necessity of adopting biosecurity in commercial and conservation flocks. The results suggest the necessity of further studies to include a greater diversity of host species from different avian orders. However, in our findings APyV presence was not associated with typical signs nor lesions, APyV infection may represent a threat to the local avian health, in commercial or conservation establishments, of native or exotic species. Distancing and the employment of biosecurity strategies is recommended, especially in the conservation of endangered species, in triage/rehabilitation centers of the native avifauna, and among commercial exotic avian pet producers, for enabling the commercial viability of the animals and to reduce the risk of avian pets acting as virus source.

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