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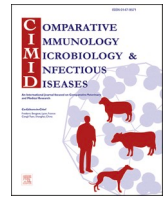
Novel adenoviruses from captive psittacine birds in Slovenia

Marko Zadravec [a], Joško Račnik [a], Brigita Slavec [a], Mónika Z. Ballmann [b], Győző L. Kaján [b], Andor Doszpoly [b], Olga Zorman-Rojs [a], Cvetka Marhold [a], Balázs Harrach [b,*]

[a] Institute of Poultry, Birds, Small Mammals and Reptiles, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000, Ljubljana, Slovenia
[b] Veterinary Medical Research Institute, Eötvös Loránd Research Network, H-1143 Budapest, Hungária krt. 21, Hungary

ABSTRACT

To assess the prevalence of adenoviruses in psittacine birds kept in Slovenia, 258 cloacal swabs were collected from different psittacine species and screened by a nested PCR with degenerate, consensus primers targeting the adenoviral DNA polymerase gene. Forty-two samples were found to be positive. By sequencing, 28 samples from 10 different parrot species were identified as the formerly described siadenovirus, psittacine adenovirus 2 (PsAdV-2). A second siadenovirus, a variant of PsAdV-5 (described earlier from Pacific parrotlet, sun parakeet, cockatiel and budgerigar) was found in seven budgerigars, two cockatiels and an amazon parrot species. A variant of Meyer's parrot adenovirus (aviadenovirus, proposed PsAdV-8) was identified in an African grey parrot and a cockatiel. Two novel atadenoviruses were revealed in cockatiel (PsAdV-9) and rose-ringed parakeet (PsAdV-10). These results support the earlier finding that many PsAdVs can cross the species barrier among psittacines, especially effectively in the case of PsAdV-2.

1. Introduction

Adenoviruses (AdVs) are classified into six genera within the family *Adenoviridae* [1]. Members of some genera are vertebrate class specific: genus *Mastadenovirus* contains only mammalian AdVs, *Aviadenovirus* contains avian AdVs, *Ichtadenovirus* has the single known fish AdV, genus *Testadenovirus* includes testudinoid (turtle) AdVs. Contrary, genera *Siadenovirus* and *Atadenovirus* contain AdVs infecting members of different vertebrate classes: frog, tortoise, bird and squamate siadenoviruses, and tortoise, bird, marsupial and ruminant atadenoviruses [1]. Birds can be infected by aviadenoviruses, atadenoviruses and siadenoviruses.

AdVs or adenovirus-like particles have been reported from many psittacine species including budgerigar (*Melopsittacus undulatus*), cockatiels (*Nymphicus hollandicus*), lovebirds (*Agapornis* spp.), rose-ringed parakeet (*Psittacula krameri*), amazon parrots (*Amazona* spp.), eclectus parrot (*Eclectus roratus*), African grey parrot (*Psittacus erithacus*), Cape parrots (*Poicephalus robustus*) and lorikeets (*Trichoglossus* spp.) [2–8]. Intranuclear inclusion bodies (IIB), typical of AdVs, have been seen in different parrot species in different internal organs, most often liver or kidney. IIB in the liver (inclusion body hepatitis) was first described by

Scott et al. [2] in a cockatiel and is the predominant pathological finding in most psittacine species [9]. In budgerigars, however, IIBs were most often observed in the kidneys and rarely in other internal organs [10, 11].

Formerly, AdV infections were diagnosed based on the typical histological lesions and/or electron microscopic findings, but classification was out of reach. Serology has also limited value in psittacines since the degree of cross-reactivity of antibodies among PsAdVs is not known. Nowadays, PCR seems to be the method of choice in PsAdV detection and classification, especially in search for novel ones. Hexon or DNA-dependent DNA polymerase gene (*pol*) targeting PCRs have been developed and are the most applied techniques in AdV screening [12, 13]. The first psittacine adenoviral *pol* sequence was gained from a Meyer's parrot (*Poicephalus meyeri*), whereas the first partial hexon sequence from Senegal parrots (*Poicephalus senegalus*) [12,13].

A PCR survey was conducted for AdVs in cloacal swabs collected from living psittacines in Slovenia. Clinical records of the AdV positive birds were examined and correlations between AdV type, bird's age, origin of host species and clinical signs were investigated.

2. Materials and methods

2.1. Sample collection

At the Clinic for Birds, Small Mammals and Reptiles of the Veterinary Faculty, University of Ljubljana, 258 psittacine birds from 23 species were sampled using cloacal swabs (Table 1). Birds were brought to the clinic as patients or for routine clinical examination. The samples were stored at −20 ºC until processing.

2.2. DNA extraction

Two mL of PBS was added to each sample and vortexed vigorously. DNA was purified from 200 µL of the suspension with the QIAamp® DNA Mini kit (Qiagen) according to the manufacturer's recommendation.

2.3. PCR and sequencing

A nested PCR with degenerate, consensus primers targeting the adenoviral *pol* was used according to the original description [13]. The following PCR primers were used. For the first amplification, forward primer polFouter (5′-TNMGNGGNGGNMGNTGYTAYCC-3′, where Y = C or T, N = A, C, G, or T, and M = A or C); reverse primer, polRouter (5′-GTDGCRAANSHNCCRTABARNGMRTT-3′, where R = A or G, M = A or C, D = A, G, or T, S = G or C, H = A, T, or C, and B = G, T, or C). For the second round, forward primer polFinner (5′-GTNTWYGAYATHT GYGGHATGTAYGC-3′, where W = A or T) and reverse primer polRinner (5′-CCANCCBCDRTTRTGNARNGTRA-3′). After electrophoresis, the PCR products were excised from the gel, purified with the Wizard PCR Preps DNA Purification System (Promega) and sent for sequencing to Macrogen DNA Sequencing Service.

2.4. Phylogenetic analysis

The virus sequences were identified using BLAST homology search at the NCBI portal. Multiple alignments were prepared using the MultAlin program. Phylogenetic calculations were conducted with the PHYLIP package using ProtDist with Categories model, then Fitch (Fitch-Margoliash method with global rearrangements); trees visualized by MEGA7. The mitochondrially encoded cytochrome c oxidase I (COX1) proteins of the discussed psittacine hosts were collected from the databank of NCBI. When they were not available from the adenovirus hosting psittacine species, we applied the COX1 from another host of the same genus. After multiple alignment using MultAlin, maximum-likelihood (PhyML) calculation was performed on the Galaxy server of the Pasteur Institute by applying evolutionary model LG and finally SH-like statistical test for branch support (https://galaxy.pasteur.fr).

3. Results

AdV was revealed from 42 samples from 258 living psittacines gained randomly by cloacal swabbing (16.3%, Table 1). Five AdV types were identified; two novel atadenoviruses, two known siadenoviruses

Table 1
List of the studied psittacine samples.

Species sampled	Sampled birds	AdV positives	positivity (%)
African grey parrot (*Psittacus erithacus*)	13	**1** (avi)	7.7
Alexandrine parakeet (*Psittacula eupatria*)	2	**1** (si)	50
amazon parrot (*Amazona* spp.)	9	**3** (2 +1 si)	33.3
Macaw (*Ara* spp.)	7	0	0
Australian king parrot (*Alisterus scapularis*)	1	**1** (si)	100
Barraband's parrot (superb parrot, *Polytelis swainsonii*)	4	0	0
black-headed parrot (*Pionites melanocephalus*)	3	0	0
budgerigar (*Melopsittacus undulatus*)	113	**11** (7 +4 si)	9.7
burrowing parrot (*Cyanoliseus patagonus*)	1	0	0
cockatiel[a] (*Nymphicus hollandicus*)	52	**17** (1 at, 1 avi, 13 +2 si)	**32.7**
cockatoo spp.	7	**3** (si)	42.9
crimson rosella (*Platycercus elegans*)	1	0	0
eastern rosellas (*Platycercus eximius*)	2	**1** (si)	50
eclectus parrot (*Eclectus roratus*)	1	0	0
lovebird (*Agapornis* spp.)	18	0	0
nanday parakeet (*Aratinga nenday*)	2	0	0
Neophema spp.	3	**1** (si)	33.3
rainbow lorikeet (*Trichoglossus moluccanus*)	1	0	0
red-crowned parakeet (kakariki, *Cyanoramphus novaezelandiae*)	2	0	0
red-winged parrot (*Aprosmictus erythropterus*)	1	**1** (si)	100
rose-ringed parakeet (*Psittacula krameri*)	12	**2** (1 at, 1 si)	16.7
Senegal parrot (*Poicephalus senegalus*)	1	0	0
sun parakeet (*Aratinga solstitialis*)	2	0	0
n = 23	n = 258	n = **42**	16.3

Bold letters show the birds found positive for AdV. The genera of the identified AdVs are abbreviated: avi, aviadenovirus; si, siadenovirus; at, atadenovirus.

[a] Cockatiel have been found most commonly positive for AdV (32.7%) among birds with more than eight samples taken per species. There were five different AdV sequences (two siadenoviruses, two atadenoviruses and one aviadenovirus) and only cockatiel was found hosting four of them.

Table 2
Psittacine adenoviruses found in the study (42 AdV sequences).

Identified virus (GenBank accession number)	Host, virus strain	Infected birds / number of birds with this AdV	Percentage in all AdV positive samples
psittacine AdV-2 (PsAdV-2; known **siadenovirus**, 28 seqs)	13 cockatiels	13/28 = 46.4%	28/42 = 66.7%
	4 budgerigars	4/28 = 14.3%	
	3 *Cacatua* sp.	3/28 = 10.7%	
	2 amazon parrots	2/28 = 7.1%	
	1 Alexandrine parakeet	1/28 = 3.6%	
	1 Australian king parrot	1/28 = 3.6%	
	1 eastern rosella	1/28 = 3.6%	
	1 *Neophema* sp.	1/28 = 3.6%	
	1 red-winged parrot	1/28 = 3.6%	
	1 rose-ringed parakeet	1/28 = 3.6%	
PsAdV-5 strain 129AM (variant of known **siadenovirus** type, 10 seqs; OK058275)	7 budgerigars, strain 129AM	7/10 = 70%	10/42 = 23.8%
	2 cockatiels	2/10 = 20%	
	1 amazon parrot	1/10 = 10%	
PsAdV-8 strain AL32 (Meyer's parrot AdV-like virus; variant of known **aviadenovirus** type, 2 seqs; OK058272)	1 African grey parrot, AL32	1/2 = 50%	2/42 = 4.8%
	1 cockatiel	1/2 = 50%	
PsAdV-9 (novel **atadenovirus**, 1 seq; OK058273)	1 cockatiel AL87	1/1 = 100%	1/42 = 2.4%
PsAdV-10 (novel **atadenovirus**, 1 seq; OK058274)	1 rose-ringed parakeet, strain 22AM	1/1 = 100%	1/42 = 2.4%

and a variant of an earlier described aviadenovirus (Table 2). One of the new atadenoviruses (2.4% of the total number of positive samples) was obtained from a rose-ringed parakeet, the second from a cockatiel. Regarding siadenoviruses, a variant of PsAdV-5 was detected ten times (23.8%): in 7 budgerigars, 2 cockatiels and an amazon parrot species. Siadenovirus PsAdV-2 was the most predominant detected in 28 cloacal swabs taken from 13 cockatiels, 4 budgerigars, 3 cockatoos (*Cacatua* sp.), 2 amazon parrot species, an Alexandrine parakeet (*Psittacula eupatria*), an Australian king parrot (*Alisterus scapularis*), an eastern rosella (*Platycercus eximius*), a *Neophema* sp., a red-winged parrot (*Aprosmictus erythropterus*) and a rose-ringed parakeet (66.7% of all the positive samples). The nucleotide (nt) sequences of the two identified aviadenoviruses revealed from African grey parrot and cockatiel were identical with each other and had 84% identity with the nt sequence of the Meyer's parrot AdV while their amino acid sequences showed 99% identity (89/90 aa). In case of three positive samples, from budgerigar, lovebird and red-winged parrot, the exact sequence of the amplicons could not be determined, as the electrophoretogram contained double or multiple peaks.

The clinical records revealed that all parrots infected with the newly described atadenoviruses or PsAdV-5 were clinically healthy (Table 3). Similarly, the cockatiel infected with the Meyer's parrot AdV-like virus was clinically healthy, too, however, the African grey parrot died shortly after presentation with signs of acute hepatic disease. Among 28 parrots infected with PsAdV-2, 10 were clinically healthy, 15 had clinical signs and in 3 cases clinical data were not available. Six of the cockatiels with PsAdV-2 had feather damaging behaviour.

To gain insight in the evolutionary origin of parrot AdVs, sampled species were grouped based on their continental origin (Table 4). Parrots from Africa had the lowest percentage of positivity with only one African grey parrot (1/32; 3.1%). Samples from hosts of South American (3/24; 12.5%), Australian (35/188; 18.6%) as well as Asian origin (3/14; 21.4%) had higher level of positivity. The highest diversity of hosts was revealed among Australian species: 7 species and 35 samples were positive for AdVs. The cockatiel was the species most infected with AdVs (17/52; 32.7%) and the only species infected with AdVs from all three genera (Table 2).

4. Discussion

AdVs have been characterized by sequencing from Senegal parrot, plum-headed parakeet (*Psittacula cyanocephala*), umbrella cockatoo (*Cacatua alba*), budgerigar, cockatiel, eastern rosella, red-crowned parakeet (*Cyanoramphus novaezelandiae*), scarlet-chested parrot (*Neophema splendida*), red-bellied parrot (*Poicephalus rufiventris*), blue-crowned conure (*Aratinga acuticaudata*), mealy parrot (*Amazona farinosa*), orange-bellied parrots (*N. chrysogaster*) and Bourke's parrot (*Neopsephotus bourkii*) [14–16] (GenBank acc. MN687905). In the present study, the presence of five AdVs was confirmed. An AdV prevalence rate of 16.3% was observed among the Slovenian parrots, whereas a survey of captive parrots in Australia (fresh droppings from 109 clinically normal parrots and 9 with feather abnormalities) from 11 aviaries has detected only 4 positives (3.4%, all in clinically normal parrots) [17]. The difference is most probably in the methodology: Hulbert et al. [17] applied a hexon-gene-targeting PCR, with primers based on fowl AdVs [12]. Thus, its target range is only the members of the genus *Aviadenovirus*, most probably not even all of them.

PsAdV-2 was detected in 28 samples. This siadenovirus has been described from plum-headed parakeet, umbrella (white) cockatoo, budgerigar, cockatiel, eastern rosella and red-crowned parakeets, orange-bellied, Bourke and scarlet-chested parrots, and recently from a moribund African grey parrot [15,18–23]. We detected PsAdV-2 in 10 species (Alexandrine and rose-ringed parakeets; amazon, Australian king and red-winged parrots; budgerigar, cockatiel, *Cacatua* sp., eastern rosella, *Neophema* sp.) thus increasing the number of known hosts by five new species (Alexandrine and rose-ringed parakeets; amazon, Australian

king and red-winged parrots). PsAdV-2 was the predominant type in Slovenia. It was detected in 10.8% of sampled parrots (28/258) and represented 2/3 (66.7%) of the detected AdVs. The highest number of PsAdV-2 detection was among cockatiels (13/52) despite more than twice as many budgerigars (4/113) screened. Ballmann and Vidovszky [15] had detected PsAdV-2 in 7/124 random samples (5.6%) of 21 species in Hungary neighbouring Slovenia (detected in 5 species: 3 budgerigars, a cockatiel, an eastern rosella, a scarlet-chested parrot and a red-crowned parakeet). The higher prevalence in our study could be due to differences in the sampling method. Ballmann and Vidovszky had sampled living and dead animals (cloaca, internal organs), while only cloacal swabs of living birds were studied by us and only from birds presented for clinical examination. Some of those samples originated from healthy breeder flocks just to widen the scale of the studied species.

PsAdV-5 (siadenovirus) was detected in budgerigars, cockatiels and an amazon parrot species. The same type (99% nt identity on the partial *pol*) has been detected already in Pacific parrotlet (*Forpus coelestis*) and sun parakeet (syn. sun conure; *Aratinga solstitialis*) [24] (GenBank

Table 3

Description of the AdV positive birds.

AdV type	Bird species	Age at sampling	Clinical signs[a]
PsAdV-2 (siadenovirus)	budgerigar	1 year	cnemidocoptes
	budgerigar	1 year	none
	2 budgerigars	juvenile	none
	cockatiel	20 years	apatia, cahexio, tracheitis
	cockatiel	19 years	tumor gl. uropigealis
	cockatiel	18 years	vomiting, candidiasis
	cockatiel	11 years	limping, hepatomegalia
	cockatiel	10 years	feather-picker
	cockatiel	7 years	egg overproduction
	cockatiel	3 years	feather-picker
	2 cockatiels	1 year	feather-pickers
	2 cockatiels	adult - unknown	feather-pickers
	2 cockatiels	adult - unknown	none
	Alexandrine parakeet	1 year	apatia, cahexio, mites
	red-winged parrot	1 year	none
	Neophema sp.	1 year	none
	eastern rosella	adult - unknown	none
	Cacatua sp.	11 years	feather-picker
	2 *Cacatua* sp.	adult - unknown	unknown
	rose-ringed parakeet	adult - unknown	none
	amazon parrot	5 years	sinusitis
	amazon parrot	2 years	unknown
	Australian king parrot	adult - unknown	none
PsAdV-5 (siadenovirus)	budgerigar	10 years	none, lipoma
	budgerigar	2 years	none (cnemidocoptes)
	4 budgerigars	1 year	none
	budgerigar	1 year	abn. feather growth
	cockatiel	3 years	none
	cockatiel	1 year	none
	amazon parrot	11 years	none, obesitas, beak overgrowth
PsAdV-8 (Meyer's parrot AdV-like aviadenovirus)	African grey parrot	adult - unknown	sudden death (hepatitis)
	cockatiel	3 years	none
PsAdV-9 (atadenovirus)	cockatiel	adult - unknown	none
PsAdV-10 (atadenovirus)	rose-ringed parakeet	adult - unknown	none

[a] None – clinically healthy

Table 4

The continental origin of parrots and the distribution of their AdV contamination.

Continent of origin	Bird species	Number of positive/ No of examined birds	Detected AdV
Africa	African grey parrot	1/13	1/32 (**3.1%**)
	lovebird	0/18	Σ 1/258 (0.4%)
	Senegal parrot	0/1	Σ AdV 1/42 (2.4%)
Asia	rose-ringed parakeet	2/12	3/14 (**21.4%**)
	Alexandrine parakeet	1/2	Σ 3/258 (1.2%)
			Σ AdV 3/42 (7.1%)
Australia	cockatiel	17/52	35/188 (**18.6%**)
	budgerigar	11/113	Σ 35/258 (13.56%)
	cockatoo spp.	3/7	Σ AdV 35/42 (83.33%)
	Australian king parrot	1/1	
	red-winged parrot	1/1	
	eastern rosella	1/2	
	Neophema spp.	1/3	
	Barraband's parrot	0/4	
	red-crown parakeet	0/2	
	crimson rosella	0/1	
	eclectus parrot	0/1	
	rainbow lorikeet	0/1	
South America	amazon parrot	3/9	3/24 (**12.5%**)
	Ara sp.	0/7	Σ 3/258 (1.16%)
	black-headed parrot	0/3	Σ AdV 3/42 (7.14%)
	nanday parakeet	0/2	
	sun parakeet	0/2	
	burrowing parrot	0/1	

MK695679), but also from a cockatiel and budgerigars [11,25,26]. In the latter cases, no sequence was obtained from the *pol* the typing was hexon based. PsAdV-5, together with PsAdV-6 (from budgerigar and rainbow lorikeet; *Trichoglossus moluccanus*), belong to the recently established species *Psittacine siadenovirus D* [1] (https://ictv.global/-filebrowser/download/7674). PsAdV-5 s were revealed from young to a 10-year-old budgerigar, kept alone without contact with other parrots. It may have a low virulence to parrots since all the birds were clinically healthy.

Only one aviadenovirus type was identified, the same type from a cockatiel and an African grey parrot. The obtained nt sequence was 83.7% identical with that of the Meyer's parrot AdV, while the aa sequence showed 98.9% identity, i.e., one amino acid difference in the partial DNA polymerase sequence [13]. Based on the high sequence identity, these strains were preliminary classified into the same type. As it was detected in three different psittacine host species already, it seems to be logical to designate it as "psittacine" instead of referring only to the first recognized host (proposed PsAdV-8). In the original case, a 23-day-old Meyer's parrot died suddenly with severe acute hepatocellular necrosis. In our case, the African grey parrot died shortly after samples were taken with 2-days lethargy, anorexia, crop stasis and biliverdinuria. Clinical signs were similar to those described in the Meyer's parrot and an enlarged, pale liver was seen on necropsy. The PsAdV-8 positive cockatiel was 3-year-old and clinically healthy, purchased as a young animal and had no contact with other parrots ever since. Based on clinical examination, the virus had only minor pathogenic impact on the cockatiel's health status, if any. Wellehan et al. [13] described the death of multiple parrots, which was observed 2 weeks after several new cockatiels had been added to the Meyer's parrot's aviary. The African grey parrot in our study originated also from a private collection of multiple parrot species, including cockatiels. The cockatiel could serve as an asymptomatic, healthy carrier of PsAdV-8, causing a spillover to other birds and a fatal disease in naïve parrot populations of other species.

We detected two novel atadenoviruses, one in cockatiel (designated PsAdV-9) and one in rose-ringed parakeet (PsAdV-10) (Fig. 1). PsAdV-9 is very similar to the sulphur-crested cockatoo (*Cacatua galerita*) AdV found in Australia [21]. Both Slovenian birds were clinically healthy adults; but data on exact age was missing. To et al. [27] reported an atadenovirus in southern mealy amazon (PsAdV-3 strain HKU1), the viral load of which positively correlated to *Chlamydophila psittaci* load in this parrot's lung. The virus has been identified only in diseased parrots with concurrent *C. psittaci* infection leading to speculation that PsAdV-3 may have caused immunosuppression among infected parrots and consequently heavy infection with *C. psittaci* and a higher chance of zoonotic transmission [27]. However, an opposite scenario is that the *C. psittaci* caused immunosuppression and this led to the multiplication of the otherwise not harmful PsAdVs. Further atadenoviruses have been identified in long-billed corella (*Cacatua tenuirostris*) and the mentioned sulphur-crested cockatoo in Australia [21], and white-eyed parakeet (*Psittacara leucophthalmus*) in Brazil [28] (Fig. 1).

Prevalence data of AdVs in parrot species depending on their continental origin showed the lowest percentage of AdV positivity in African parrots (3.1%; only a single bird, an African grey parrot, was found to be infected), while higher percentage was detected in South American (12.5%), Australian (18.6%) and Asian (21.4%) species (Table 4). However, the last value originates from only three birds (rose-ringed and Alexandrine parakeets). In South American psittacines, a single (amazon parrot) species proved positive among six. Diversity of parrots is the greatest on the Australian continent. Australian species were represented with the highest number (12), the most individuals sampled (188) and the highest number of positive species (7). The highest positivity rate was observed also in an Australian species (cockatiel, 32.7%) the only species in which representatives from all three genera of avian AdVs were found. It is hypothesized that siadenoviruses co-evolved with Australian parrots since some time and switched to hosts originating from other continents relatively recently. Especially, PsAdV-2 and PsAdV-5 partial sequences from different bird species are highly similar, respectively, reflecting a short time for divergence in the new host. Cockatiels were more often recorded with non-fatal diseases and could serve as asymptomatic carriers for different types besides PsAdV-8 (Fig. 2).

AdV can cause hepatic disorders, haemorrhagic enteritis, yellowish urine and faeces, weight loss, ruffled feathers, signs of respiratory diseases or sudden death [25,29,30]. Due to the diversity of clinical signs and AdV types, no exact correlation among types and specific pathological conditions has been determined. At the first detection of PsAdV-2 in diseased plum-headed parakeet and umbrella cockatoo [18], it was supposed to be the cause of mortality in both. The crossing of species barrier also suggests an enhanced pathogenicity. In our study, a correlation seems to exist between PsAdV-2 positivity and feather damaging behaviour in cockatiels. Feather damaging behaviour in parrots is around 10% [31] and is particularly common in the African grey parrot, cockatoos and eclectus parrot [32]. Cockatiel seems less affected: 7.6% in the Italian population [33]. Causes of this behavioural problem include socio-environmental, neurobiological, genetic and medical origins. Since liver is one of the organs most affected by AdVs and hepatic diseases are one of the possible causes of feather damaging behaviour [32], connection may be presumed. But feather damaging behaviour also cause immunosuppression with chronically elevated corticosterone levels [34] enabling PsAdV-2 replication.

A lipometabolic disorder, disseminated xanthogranulomatosis, appearing simultaneously in several internal organs, has been recently described in red-crowned parakeet and a possible association with a concurrent PsAdV-2 infection [35]. AdV infections that trigger lipometabolic diseases (obesity) have been supposed in humans and chickens thus a similar effect in the PsAdV-2 infected different psittacines should be monitored in the future.

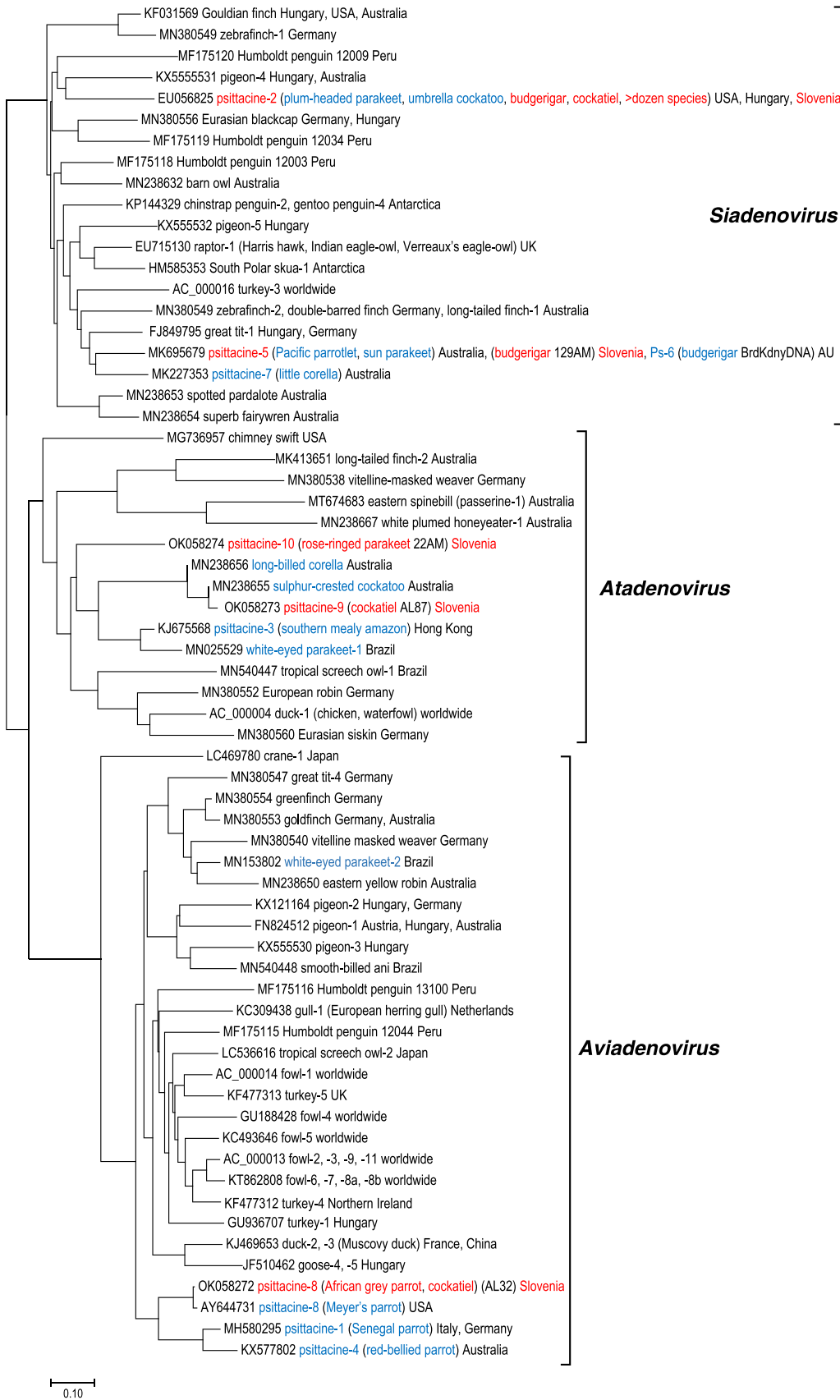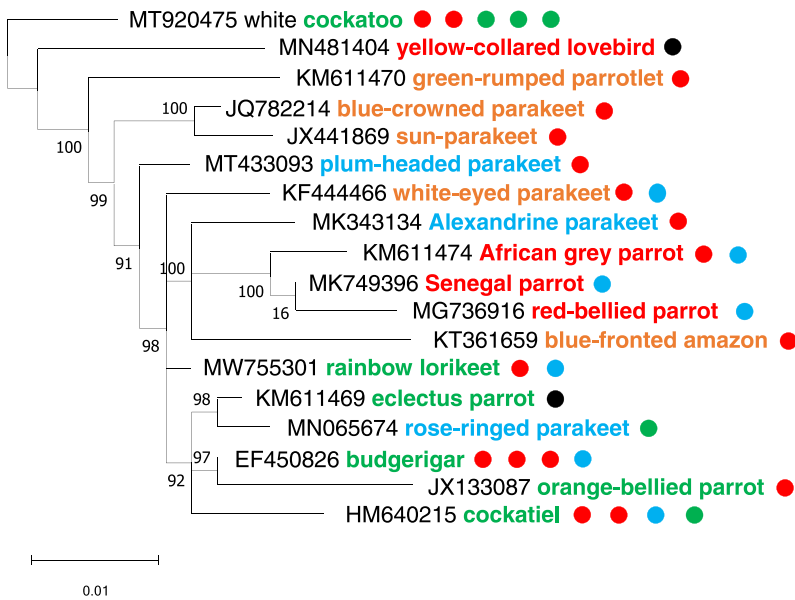Recently, fulminant hepatitis cases were reported from children

Fig. 1. Distance matrix analysis of partial DNA polymerase sequences of avian adenoviruses. ProtDist with Categories model, Fitch with global rearrangements, non-routed calculation. Psittacine adenoviruses recovered by the present work from Slovenian samples are shown by red letters followed by the strain identifier. Psittacine adenoviruses described in other countries are shown by blue letters followed by the country of origin of the sample. The three genera containing bird adenoviruses are shown. Branch lengths measured in the number of substitutions per site.

Fig. 2. Phylogenetic (PhyML) tree of the mitochondrially encoded cytochrome c oxidase I (COX1) gene of the discussed psittacine hosts when available in the databank of NCBI. The numbers at the nodes show the SH-like branch support. The continental origin of the psittacines is shown by the colour of the letters, red: Africa, blue: Asia, green: Australia, orange: South America. The identified infecting adenovirus types are represented by corresponding number of dots, red: siadenovirus, blue: aviadenovirus, green: atadenovirus. black: not typed, only morphologically identified adenovirus. White (umbrella) cockatoo (*Cacatua alba*) represents also the sulphur-crested cockatoo (*C. galerita*), long-billed corella (*C. tenuirostis*), and little corella (*C. sanguinea*). The scarlet-chested parrot (*N. splendida*) and other *Neophema* spp. are represented by orange-bellied parrot (*N. chrysogaster*); Pacific parrotlet (*Forpus coelestis*) by green-rumped parrotlet (*F. passerinus*); Cape parrot (*Poicephas robustus*) and Meyer's parrot (*P. meyeri*) by Senegal parrot (*P. senegalus*); amazon parrot (Amazona spp.), Tucumán amazon (*A. tucumana*), and southern mealy amazon (*A. farinose*) by blue-fronted amazon (*A. aestiva*).

under 5 years of age, and an association with human AdV and adeno-associated virus 2 was hypothesized [36]. It would be useful to study a similar possibility in psittacine birds, first by recovering psittacine dependoviruses either by consensus PCR or by phi29 enzyme from the sick birds.

5. Conclusions

Two novel atadenoviruses were found in psittacine birds kept in Slovenia, and a variant of Meyer's parrot adenovirus (an aviadenovirus). Two earlier described siadenoviruses were found in 13 different psittacine species. Psittacine adenoviruses proved capable of easily crossing the species barrier, which is an uncommon phenomenon among the adenoviruses. The psittacine adenoviruses may be responsible for several diseases in parrots including feather damaging behaviour and mortality.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] M. Benkő, K. Aoki, N. Arnberg, A.J. Davison, M. Echavarría, M. Hess, M.S. Jones, G. L. Kaján, A.E. Kajon, S.K. Mittal, et al., ICTV virus taxonomy profile: *Adenoviridae* 2022, J. Gen. Virol. 103 (2022), 001721, https://doi.org/10.1099/jgv.0.001721.

[2] P.C. Scott, R.J. Condron, R.L. Reece, Inclusion body hepatitis associated with adenovirus-like particles in a cockatiel (Psittaciformes; *Nypmhicus hollandicus*), Aus. Vet. J. 63 (1986) 337–338, https://doi.org/10.1111/j.1751-0813.1986.tb02879.x.

[3] D.A. Pass, Inclusion bodies and hepatopaties in psittacines, Avian Pathol. 16 (1987) 581–597, https://doi.org/10.1080/03079458708436408.

[4] E.R. Jacobson, C. Gardiner, S. Clubb, Adenovirus-like infection in white-masked lovebirds (*Agapornis personata*), J. Ass. Avian Vet. 1 (1989) 32–34.

[5] J.C. Gómez-Villamandos, E. Mozos, M.A. Sierra, J. Pérez, A. Mendez, Inclusion body containing adenovirus-like particles in the intestine of a psittacine bird affected by inclusion body hepatitis, J. Wildl. Dis. 28 (1992) 319–322, https://doi.org/10.7589/0090-3558-28.2.319.

[6] A. Ramis, M.J. Marlasca, N. Majo, L. Ferrer, Inclusion body hepatitis (IBH) in a group of eclectus parrots (*Eclectus roratus*), Avian Pathol. 21 (1992) 165–169, https://doi.org/10.1080/03079459208418831.

[7] R. Droual, P.R. Woolcock, R.W. Nordhausen, S.D. Fitzgerald, Inclusion body hepatitis and hemorrhagic enteritis in two African grey parrots (*Psittacus erithacus*) associated with adenovirus, J. Vet. Diagn. Invest 7 (1995) 150–154, https://doi.org/10.1177/104063879500700125.

[8] J.T. Mackie, D. Black, H. Prior, Enteritis associated with adenovirus-like particles in captive lorikeets, Aust. Vet. J. 81 (2003) 293–295, https://doi.org/10.1111/j.1751-0813.2003.tb12578.x.

[9] H. Weissenböck, A. Fuchs, Histological and ultrastructural characterization of hepatic intranuclear inclusion bodies in psittacine birds and pigeons, Avian Pathol. 24 (1995) 507–521, https://doi.org/10.1080/03079459508419090.

[10] F. Mori, A. Touchi, T. Suwa, C. Itakura, A. Hashimoto, K. Hirai, Inclusion bodies containing adenovirus-like particles in the kidneys of psittacine birds, Avian Pathol. 18 (1989) 197–202, https://doi.org/10.1080/03079458908418591.

[11] H. Katoh, K. Ohya, M. Kubo, K. Murata, T. Yanai, H. Fukushi, A novel budgerigar-adenovirus belonging to group II avian adenovirus of *Siadenovirus*, Virus Res. 144 (2009) 294–297, https://doi.org/10.1016/j.virusres.2009.04.012.

[12] R. Raue, H. Gerlach, H. Müller, Phylogenetic analysis of the hexon loop 1 region of an adenovirus from psittacine birds supports the existence of a new psittacine adenovirus (PsAdV), Arch. Virol. 150 (2005) 1933–1943, https://doi.org/10.1007/s00705-005-0578-x.

[13] J.F.X. Wellehan, A.J. Johnson, K.S. Latimer, K. Bischoff, M. Lafortune, E. R. Jacobson, Identification and initial characterization of an adenovirus associated with fatal hepatic and lymphoid necrosis in a Meyer's parrot (*Poicephalus meyeri*), J. Avian Med. Surg. 19 (2005) 191–197.

[14] D. Lüschow, C. Prusas, M. Lierz, H. Gerlach, D. Soike, H.M. Hafez, Adenovirus of psittacine birds: investigation on isolation and development of a real-time polymerase chain reaction for specific detection, Avian Pathol. 36 (2007) 487–494, https://doi.org/10.1080/03079450701691260.

[15] M.Z. Ballmann, M.Z. Vidovszky, Széles gazdaspektrumú psittacin adenovírus (PsAdV-2) kimutatása különböző papagájfajok hazai egyedeiben. [Detection of broad-host range psittacine adenovirus (PsAdV-2) in representatives of different parrot species (in Hungarian)] Magy. Állatorvosok 135 (2013) 78–87.

[16] M. Sutherland, S. Sarker, P.K. Vaz, A.R. Legione, J.M. Devlin, P.L. Macwhirter, P. L. Whiteley, S.R. Raidal, Disease surveillance in wild Victorian cacatuids reveals co-infection with multiple agents and detection of novel avian viruses, Vet. Microbiol. 235 (2019) 257–264, https://doi.org/10.1016/j.vetmic.2019.07.012.

[17] C.L. Hulbert, A. Chamings, K.A. Hewson, P.A. Steer, M. Gosbell, A. H. Noormohammadi, Survey of captive parrot populations around Port Philip Bay, Victoria, Australia, for psittacine beak and feather disease virus, avian

polyomavirus and psittacine adenovirus, Aust. Vet. J. 93 (2015) 287–292, https://doi.org/10.1111/avj.12350.

[18] J.F.X. Wellehan, C.B. Greenacre, G.J. Fleming, M.D. Stetter, A.L. Childress, S.P. Terrell, Siadenovirus infection in two psittacine bird species, Avian Pathol. 38 (2009) 413–417, https://doi.org/10.1080/03079450903183660.

[19] D.N. Phalen, J. Agius, F.F. Vaz, J.-S. Eden, L.C. Setyo, S. Donahoe, A survey of a mixed species aviary provides new insights into the pathogenicity, diversity, evolution, host range, and distribution of psittacine and passerine adenoviruses, Avian Pathol. 48 (2019) 437–443, https://doi.org/10.1080/03079457.2019.1617835.

[20] N. Yang, J. McLelland, D.J. McLelland, J. Clarke, L. Woolford, P. Eden, D.N. Phalen, Psittacid Adenovirus-2 infection in the critically endangered orange-bellied parrot (*Neophema chrysogastor*). A key threatening process or an example of a host-adapted virus? PloS One 14 (2019), e0208674 https://doi.org/10.1371/journal.pone.0208674.

[21] F.F. Vaz, T.F. Raso, J.E. Agius, T. Hunt, A. Leishman, J.S. Eden, D.N. Phalen, Opportunistic sampling of wild native and invasive birds reveals a rich diversity of adenoviruses in Australia, Virus Evol. 6 (2020) veaa024, https://doi.org/10.1093/ve/veaa024.

[22] A. Athukorala, D.N. Phalen, A. Das, K.J. Helbig, J.K. Forwood, S. Sarker, Genomic characterisation of a highly divergent siadenovirus (Psittacine siadenovirus F) from the critically endangered orange-bellied parrot (*Neophema chrysogaster*), Viruses 13 (2021) 1714, https://doi.org/10.3390/v13091714.

[23] A. Surplis, J. Dill-Okubo, B. Harrach, T. Waltzek, K. Subramaniam, Genomic characterization of psittacine adenovirus 2, a siadenovirus identified in a moribund African grey parrot (*Psittacus erithacus*), Arch. Virol. 167 (2022) 911–916, https://doi.org/10.1007/s00705-021-05341-2.

[24] N.L. Gottdenker, C.R. Gregory, M.B. Ard, W.W. Lorenz, R.A. Nilsen, B.W. Ritchie, Histopathologic changes, ultrastructure, and molecular characterization of an adenovirus in a sun conure (*Aratinga solstitialis*), Avian Dis. 63 (2019) 531–538, https://doi.org/10.1637/aviandiseases-d-19-00080.

[25] E. Cassmann, B. Zaffrano, Q. Chen, G. Li, J. Haynes, Novel siadenovirus infection in a cockatiel with chronic liver disease, Virus Res 263 (2019) 164–168, https://doi.org/10.1016/j.virusres.2019.01.018.

[26] A.J. Gall, E.R. Burrough, J. Zhang, D.R. Magstadt, Y. Wannarat, G.W. Stevenson, R.J. Derscheid, P. Piñeyro, Y. Zheng, L. Ganwu, et al., Identification and correlation of a novel siadenovirus in a flock of budgerigars (*Melopsittacus undulates*) infected with *Salmonella Typhimurium* in the United States, J. Zoo. Wildl. Med 51 (2020) 618–630, https://doi.org/10.1638/2019-0083.

[27] K.K.W. To, H. Tse, W.-M. Chan, G.K.Y. Choi, A.J.X. Zhang, S. Sridhar, S.C.Y. Wong, J.F.W. Chan, A.S.F. Chan, P.C.Y. Woo, et al., A novel psittacine adenovirus identified during an outbreak of avian chlamydiosis and human psittacosis: zoonosis associated with virus-bacterium coinfection in birds, PLoS Negl. Trop. Dis. 8 (2014), e3318, https://doi.org/10.1371/journal.pntd.0003318.

[28] A.M. Duarte, J.M.F. Silva, C.R. Brito, D.S. Teixeira, F.L. Melo, B.M. Ribeiro, T. Nagata, F.S. Campos, Faecal virome analysis of wild animals from Brazil, Viruses 11 (2019), E803, https://doi.org/10.3390/v11090803.

[29] S. Das, K. Fearnside, S. Sarker, J.K. Forwood, S.R. Raidal, A novel pathogenic adenovirus from red-bellied parrots (*Poicephalus rufiventris*) unveils deep recombination events among avian host lineages, Virology 502 (2017) 188–197, https://doi.org/10.1016/j.virol.2016.12.031.

[30] A. Milani, G. Zamperin, A. Fusaro, A. Salviato, L. Bano, L. Zandona, R. Brunetta, I. Monne, Complete genome sequence of psittacine adenovirus 1, identified in *Poicephalus senegalus* in Italy, Microbiol. Resour. Announc 7 (2018) e01037–18, https://doi.org/10.1128/mra.01037-18.

[31] H.M. Grindlinger, E. Ramsay, Compulsive feather picking in birds, Arch. Gen. Psychiatry 48 (1991) 857, https://doi.org/10.1001/archpsyc.1991.01810330081012.

[32] Y.R.A. Van Zeeland, B.M. Spruit, T.B. Rodenburg, B. Riedstra, Y.M. Van Hierden, B. Buitenhuis, S.M. Korte, J.T. Lumeij, Feather damaging behaviour in parrots: a review with consideration of comparative aspects, Appl. Anim. Behav. Sci. 121 (2009) 75–95.

[33] P. Costa, E. Macci, L. Tomassone, F. Ricceri, E. Bollo, F.E. Scaglione, M. Tarantola, M. De Marco, L. Prola, D. Bergero, et al., Feather picking in pet parrots: sensitive species, risk factors and ethological evidence, Ital. J. Anim. Sci. 15 (2016) 473–480.

[34] P. Costa, E. Macci, E. Valle, M. De Marco, D.M. Nucera, L. Gasco, A. Schiavone, An association between feather damaging behavior and corticosterone metabolite excretion in captive African grey parrot (*Psittacus erithacus*), PeerJ 4 (2016), e2462, https://doi.org/10.7717/peerj.2462.

[35] C. Konicek, K. Heenemann, K. Cramer, T.W. Vahlenkamp, V. Schmidt, Case series of disseminated xanthogranulomatosis in red-crowned parakeets (*Cyanoramphus novaezelandiae*) with detection of psittacine adenovirus 2 (PsAdV-2). Animals (12), 2316, https://doi.org/10.3390/ani12182316.

[36] World Health Organization, Acute hepatitis of unknown aetiology in children - Multi-country. Disease Outbreak News 27 May 2022, ⟨https://www.int/emergencies/disease-outbreak-news/item/2022-DON389⟩.