Virus Research 263 (2019) 164–168

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short Communication

Novel siadenovirus infection in a cockatiel with chronic liver disease

Eric Cassmann[a,*], Bianca Zaffarano[b], Qi Chen[c], Ganwu Li[c], Joseph Haynes[a]

[a] Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[b] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[c] Veterinary Diagnostic & Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

ARTICLE INFO

Keywords:
Chronic hepatopathy
Adenovirus
Cockatiel
Psittacine siadenoviruses
Pathology
PsAdV-5

ABSTRACT

A 15-year-old female cockatiel (*Nymphicus hollandicus*) undergoing long term management for hepatopathy died and underwent necropsy. Microscopic findings were consistent with chronic liver disease characterized by distorted hepatic architecture, fibrosis and biliary proliferation. The additional finding of large intranuclear inclusion bodies within hepatocytes and renal tubular epithelium prompted diagnostic next generation sequencing. The assembled sequences isolated from pooled kidney and liver were related to siadenoviruses. The genus *Siadenovirus*, within the family *Adenoviridae,* includes several species of viruses that pathogenically infect avian species including hemorrhagic enteritis virus of turkeys and marble spleen virus of pheasants. Siadenoviruses have previously been reported in seven psittacine species: a plum-headed parakeet (*Psittacula cyanocephala*), an umbrella cockatoo (*Cacatua alba*) budgerigars (*Melopsittacus undulates*), an eastern rosella (*Platycercus eximius*), a scarlet chested parrot (*Neophema splendida*), a cockatiel (*Nymphicus hollandicus),* and a red-crowned parakeet (*Cyanoramphus novaezelandiae*). This report describes a novel siadenovirus in a cockatiel that is highly identical to budgerigar adenovirus 1 and distinct from PsAdV-2 in cockatiels. We report the clinical pathologic, gross, and histopathologic findings in a cockatiel with chronic hepatitis and a novel siadenovirus, PsAdV-5. The sequencing data is presented with a phylogenetic analysis.

Adenoviruses are non-enveloped dsDNA viruses and belong in the family *Adenoviridae*. There are three genera within *Adenoviridae* that contain virus species known to infect birds: *Atadenovirus*, *Aviadenovirus* and *Siadenovirus* (Harrach et al., 2011). Formerly identified as Group II aviadenoviruses, the genus *Siadenovirus* includes several viruses historically recognized for their pathologic impact; these include hemorrhagic enteritis virus (HEV) of turkeys and marble spleen disease virus of pheasants (Dhama et al., 2017; Fitzgerald and Reed, 1989). These viruses were classified and named as siadenoviruses due to the presence of sialidase genes distinguishing them from the other genera.

Recognized species of adenoviruses affecting psittacines belong to the genera *Atadenovirus* and *Aviadenovirus* (Das et al., 2017; Lüschow et al., 2007; Raue et al., 2005; To et al., 2014). To date, only partial genomic sequences of siadenoviruses have been reported in psittacines, precluding recognition of a new species within the genus *Siadenovirus* (Harrach et al., 2011). Case reports of psittacine siadenovirus infections include a plum-headed parakeet (*Psittacula cyanocephala*), an umbrella cockatoo (*Cacatua alba*), budgerigars (*Melopsittacus undulates*), an eastern rosella (*Platycercus eximius*), a scarlet chested parrot (*Neophema splendida*), a cockatiel (*Nymphicus hollandicus)*, and a red-crowned parakeet (*Cyanoramphus novaezelandiae*) (Ballmann and Vidovszky, 2013; Katoh et al., 2009; Wellehan et al., 2009). Herein we describe a novel siadenovirus in a cockatiel, and refer to the virus exemplar as psittacine adenovirus 5 (PsAdv-5) isolate IA17-frag.

A 15-year-old female cockatiel weighing 109 g presented to the Iowa State University Exotic Service for a 24-h period of lethargy, inappetence, refusing to drink water, and sitting fluffed on bottom of the cage. She was fed a diet of commercial pellets and seeds as a snack. She shared a house with five other birds, two cockatiels, two budgerigars (*Melopsittacus undulates*), and a sun conure (*Aratinga solstitialis*), in the same room.

Initial laboratory testing consisted of a complete blood count (CBC), serum biochemical profile, full body radiographs, ultrasound of the coelomic cavity, and a crop/fecal gram stain. Abnormalities on the CBC included leukocytosis, heterophilia, and a slight monocytosis. Chemical alterations included an elevated AST, elevated bile acid (fasting), elevated creatine kinase, and hypoproteinemia. Radiography and sonography indicated severe generalized hepatomegaly. Oral/fecal gram

* Corresponding author.
E-mail address: cassmann@iastate.edu (E. Cassmann).

https://doi.org/10.1016/j.virusres.2019.01.018
Received 11 October 2018; Received in revised form 31 December 2018; Accepted 31 January 2019
Available online 31 January 2019
0168-1702/

stain revealed numerous budding yeasts. Empirical supportive treatment was commenced with injectable doxycycline hyclate[1], tube feeding[2], itraconazole[3] and hospitalization.

The bird responded to treatment, but re-presented nine months later for lethargy, abnormal behavior, and weight loss (109 g down to 101 g). At this time the bile acids were over measurable limits, and the bird was hypocalcaemic and hyperphosphatemic. Multiple blood chemistries were unable to be reported on the analyser[4] presumptively due to lipemia. Radiographs yielded static severe hepatomegaly, displacing the proventriculus dorsally. The bird was hospitalized and cochicine[5] and ursodiol[6] were administered to palliate unspecified hepatic disease. Four days later the bird died.

A necropsy was performed following consent from the owners. The body condition was judged to be adequate. Two focal 5 mm diameter hemorrhages were detected in the subcutis of the left axillary area of the caudolateral breast. The liver was slightly enlarged, somewhat nodular and mottled green/brown. There were also several adhesions of the abdominal air sac to the liver capsule.

Microscopically, there were multiple bands of fibrous tissue coursing throughout the parenchyma, which were associated with multiple foci of biliary proliferation and distorted the lobular architecture of the liver (Fig. 1). There were several aggregates of enlarged hepatocytes with large vacuoles and numerous individual necrotic hepatocytes scattered throughout. In addition, multiple hepatocytes contained large intranuclear homogeneous basophilic to amphophilic inclusion bodies that effaced nuclear architecture (Fig. 1). In addition to the microscopic changes in the liver, there were intranuclear inclusion bodies and scattered epithelial cell necrosis in renal tubular epithelium.

Frozen samples of liver and kidney were submitted for Next Generation Sequencing (NGS) analysis to detect any viral pathogens, especially those that would produce the intranuclear inclusions observed in the hepatocytes and renal tubular epithelium.

For NGS analysis, the nucleic acids (DNA /RNA) were extracted and purified as follows. The liver and kidney were homogenized together into a 10% (wt/vol) solution with Earle's balanced salt solution,[7] and total DNA/RNA was extracted in duplex from the tissue homogenate[8] (Chen et al., 2018). For RNA sequencing library preparation, complimentary (c) DNA libraries were constructed as previously described with one of the duplex DNA/RNA extractions (Zhang et al., 2017). Ribosomal RNA was depleted using a commercial kit[9] followed by fragmentation. First strand cDNA was synthesized with random primers and reverse transcriptase. After removal of the RNA template, double stranded cDNA was synthesized with a replacement strand. Next, the 3′ ends were adenylated and adapters were ligated. The DNA was cleaned two more times with a commercial kit.[10] The DNA fragments with adapter molecules at both ends were enriched by PCR amplification. The other DNA/RNA extract from the duplex extraction was used directly for DNA sequencing library preparation using a commercial kit.[11]

The DNA and RNA sequencing libraries were normalized and then were sequenced on MiSeq® (Illumina, San Diego, CA) with a 300 cycle MiSeq® Reagent Micro Kit V2 (Illumina, San Diego, CA) and 150-bp end reads. The resulting raw data were pre-filtered by removing adaptors, trimming low quality ends, deleting sequences shorter than 36

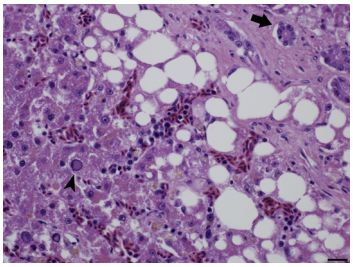

Fig. 1. Photomicrograph of liver. An intranuclear inclusion body within a hepatocyte is indicated by the arrowhead. The inclusion disrupts the normal nuclear architecture by peripheralizing chromatin and the nucleolus. Biliary proliferation (arrow) is seen within a prominent band of fibrosis. Hematoxylin and eosin. Bar, 20 µm.

nucleotides, and finally, performing a FastQC sequence quality analysis (Bolger et al., 2014). The clean nucleotide sequences were classified with Kraken v0.15.5-beta (Wood and Salzberg, 2014). The sequences were organized taxonomically, and candidate reads were identified based on their relevance to the case (capable of producing the observed lesions). These candidate reads were algorithmically assembled by SPAdes (v3.5.0) yielding contiguous *de novo* sequences. The three longest and non-overlapping contiguous segments assembled were composed of 4823, 1543 and 916 nucleotides. The 4823 nucleotide segment (GenBank MH507070) was the cleanest read following bioinformatics analysis, and it was subjected to BLASTn analysis (Morgulis et al., 2008; Zhang et al., 2000). This segment contained the complete hexon gene as well as complete pVI, pVII, pX genes and partial sequences of the penton and protease genes.

Comparative sequence analysis of the 4823-nt contiguous segment using BLASTn revealed 99% identity and 10% query coverage with a partial coding sequence of the hexon gene (528-nt) isolated from a budgerigar, budgerigar adenovirus 1 (AB485763). The 4823-nt contiguous sequence also had 76% and 75% homology and 95% and 93% query coverage with the South Polar skua adenovirus 1 (HM585353) and raptor adenovirus 1 (EU715130.4), both siadenoviruses.

The results from sequencing underwent phylogenetic analysis. Nine other adenoviruses with coverage of the hexon gene were selected from the GenBank database and aligned with the calculated hexon protein from the 4823-nt contiguous segment of PsAdV-5 isolate IA17-frag (Morgulis et al., 2008). One of the sequences in the analysis did not include coverage of the entire gene (budgerigar adenovirus 1), and therefore, it was not equal in length to the other analyzed sequences. Alignment was carried out in MEGA7 using the ClustalW method (Thompson et al., 1994). The Neighbor-Joining method was used to infer the evolutionary history of selected amino acid sequences (Saitou and Nei, 1987). The bootstrap method was applied (5000 replicates) to determine the percentage of consensus taxa clustering together (Felsenstein, 1985). The p-distance method was used to compute the evolutionary distances for the phylogenetic tree (Nei and Kumar, 2000). Overall, the analysis involved 10 amino acid sequences. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

The evolutionary relationships of the hexon proteins from selected adenoviruses are displayed in a phylogenetic tree (Fig. 2). The final dataset for the phylogenetic analysis contained 798 amino acid positions. In 5000 bootstrap replicates, the budgerigar adenovirus 1 and PsAdV-5 isolate IA17-frag clustered together 100% of the time. Budgerigar adenovirus 1 has 0.12 amino acid differences per site compared to our PsAdV-5 cockatiel isolate.

Other reported psittacine adenoviruses and their available sequencing data are listed in Table 1. In order to compare PsAdV-5 to the DNA polymerase gene of these other psittacine siadenoviruses, we performed Sanger sequencing on amplicons derived from a DNA polymerase specific PCR probe. Briefly, four 10-micron thick formalin-fixed paraffin-

[1] Vibravenos, Pfizer, New York City, NY, USA.
[2] Harrison's Recovery Formula, Harrison's Bird Foods, 7108 Crossroads Blvd. Suite 325, Brentwood, TN, USA.
[3] Sporonox, Janssen Pharmaceuticals, Inc., Titusville, NJ, USA.
[4] Colchicine, Becton,Dickinson, Sandy, UT, USA.
[5] Actigall, Watson Pharma Private Limited, Verna, Salcette Goa, INDIA.
[6] Abaxsis Vetscan, Allied Analytic, LLC.Tampa, FL, USA.
[7] Earle's balanced salt solution, Sigma-Aldrich, St. Louis, MO, USA.
[8] MagMAX™ Pathogen RNA/DNA Kit, Catalog #:4462359.
[9] Agencourt RNAClean XP, Beckman Coulter Brea, CA, USA.
[10] Agencourt AMPure XP, Beckman Coulter Brea, CA, USA.
[11] Nextera XT DNA Library Preparation Kit, Illumina.

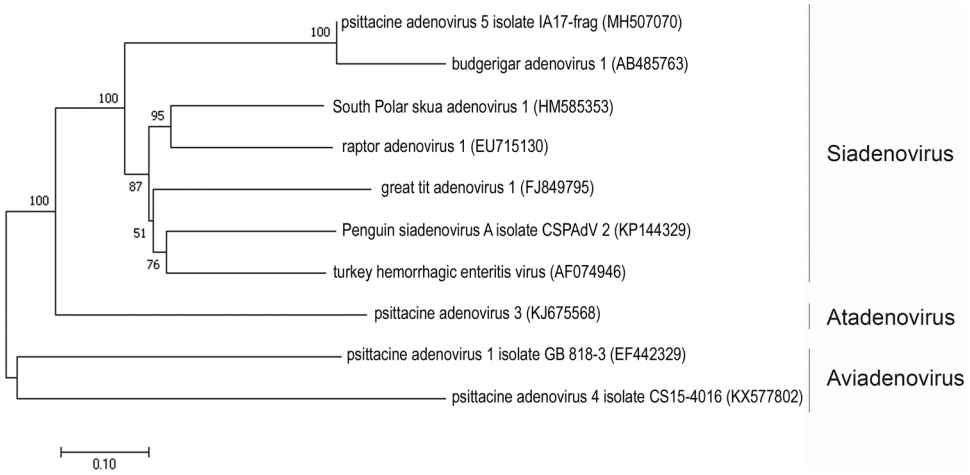

Fig. 2. The phylogenetic tree displays the evolutionary history of ten adenoviruses aligned to the hexon protein of psittacine adenovirus 5 (PsAdV-5) isolate IA17-frag. PsAdV-5 clusters together with other siadenoviruses. All viral sequences were trimmed to the full hexon gene coding sequence of PsAdV-5. Budgerigar adenovirus 1 was a partial coding sequence; therefore, the analysis includes a non-equal length sequence. The nucleotide sequences were translated, and the relationships of the protein sequences were inferred with the Neighbor-Joining method. Numbers next to the branches indicate the percentage of replicate trees in which taxa clustered together in 5000 replicate bootstrap tests. The branches are drawn to scale with the length representing the number of amino acid differences per site. Analyses were conducted in MEGA7.

embedded (FFPE) tissue scrolls were submitted for tissue extraction using a commercial extraction kit[12] . A nested primer protocol for the DNA polymerase gene of adenoviruses was used to amplify the extracted DNA (Wellehan et al., 2009, 2004). Samples were processed in duplicate. The outside and inside forward and reverse PCR amplicons were all submitted for Sanger sequencing. The longest resulting sequence had clean and discernible base calls. The overlapping segments of the sequences were highly identical. The final consensus sequence contained 441 nucleotides.

The 441-nt sequence had partial coverage of the adenovirus DNA-polymerase gene. BLASTn alignment analysis (Altschul et al., 1997) revealed 85–99% query coverage and 68–71% identity with raptor adenovirus 1 (EU715130), hemorrhagic enteritis virus (AF074946), great tit adenovirus 1 (FJ849795), and a penguin siadenovirus (KP144329). Approximately 50% of our sequence overlapped with the entire published 269-nt sequences of psittacine adenovirus 2 isolates (KC248187, KC248188, KC248189, KC248190, KC248191, KC248192, EU056825). Throughout this area of overlap (269-nt), these sequences were approximately 70% identical to PsAdV-5 isolate IA17-frag. The corresponding budgerigar adenovirus 1 DNA polymerase gene sequence is not available in Genbank.

Siadenoviruses have been recognized in many avian species including turkeys, pheasants, budgerigars, raptors, South Polar skuas, Antarctic penguins (KP144329), Gouldian finches, great tits, a Harris hawk, a Bengal eagle owl, a Verreaux's eagle owl, pigeons, an eastern rosella, a scarlet chested parrot, a cockatiel, a red-crowned parakeet, a plum-headed parakeet and an umbrella cockatoo (Ballmann and Vidovszky, 2013; Ballmann and Harrach, 2016; Dhama et al., 2017; Joseph et al., 2014; Katoh et al., 2009; Kovács and Benkő, 2009; Park et al., 2012; Wellehan et al., 2009; Zsivanovits et al., 2006). Many of these siadenovirus infections lack a description of histopathologic lesions in their respective case report. In the cockatiel of the present case, the main histologic lesions were in the liver and kidneys. This is similar to and different from other siadenovirus associated lesions. In turkeys infected with HEV, the major lesion is acute fibrinonecrotic and hemorrhagic enteritis which differentiates it from the present case; however, large intranuclear viral inclusion bodies in hepatocytes and renal tubular epithelium are sometimes observed in turkeys with HEV (Dhama et al., 2017). Raptors with siadenovirus infections had severe necrotizing hepatitis with intranuclear inclusion bodies that were also found in the kidneys and spleens of some animals (Zsivanovits et al., 2006). Gouldian finches with siadenovirus infection have lymphocytic interstitial nephritis and renal tubular necrosis with intranuclear inclusion bodies (Joseph et al., 2014). Similar to siadenoviruses in Gouldian finches, we also observed renal tubular necrosis and intranuclear inclusions within renal tubular epithelium; however, lymphocytic interstitial nephritis was absent. Interestingly, a study of siadenovirus infections in pigeons determined that virus was more easily detectable in the lung and kidneys compared to liver samples (Ballmann and Harrach, 2016). There appears to be host specific differences in viral distribution and organ tropism among different siadenoviruses. Overall, the distribution of lesions indicates multisystemic involvement with variable tropism for the liver, kidneys, intestines, lungs or spleen.

In the case reports of other psittacines with detectable siadenoviruses, lesions are inconsistent between species. Similar to our case, one budgerigar had hepatocellular necrosis, and three budgerigars had intranuclear inclusion bodies within renal tubular epithelium (Katoh et al., 2009). Additionally, the budgerigars had foci of inflammatory cells in their livers and kidneys. Another case of a siadenovirus in a plum-headed parakeet described concurrent bacterial coelomitis and sepsis after a biopsy procedure (Wellehan et al., 2009). Renal and hepatic intranuclear inclusions were not reported in that case. The same case report also describes identification of siadenovirus PCR products from an umbrella cockatoo with mononuclear meningoencephalitis. The association of these findings is unclear; in the present case, brain was not evaluated.

The finding of chronic liver disease is not typically described in reported cases of siadenovirus infections. The liver injury in our cockatiel was characterized by fibrosis, biliary proliferation, hepatocellular degeneration and necrosis. Hepatocytes frequently contained intranuclear inclusions. We cannot definitively conclude that siadenovirus infection was the cause of the chronic changes in the present case; the cockatiel had clinicopathologic abnormalities indicating hepatopathy one year prior to its death. It is possible the cockatiel had an unrelated liver disease prior to contracting a siadenovirus which acutely exacerbated a pre-existing chronic hepatopathy. The owner of the cockatiel had several other pet psittacines that could have served as a source of infection. The fulfilment of Koch's postulates is required to repudiate or substantiate a causative association between this siadenovirus and hepatitis/nephritis in cockatiels. In this case, a live virus could not be recovered for further investigation. Cockatiels that die after a history of non-specific hepatopathy or nephropathy that also have epithelial intranuclear inclusion bodies should prompt the pathologist to consider a siadenoviral infection. Cases identified as such should be further investigated and reported to establish the wider relevance of siadenoviruses in cockatiels.

Next generation sequencing was performed in this case. The longest non-overlapping contiguous segment assembled was composed of 4823 nucleotides (MH507070). This segment contained a complete sequence for the hexon gene which had 99% identity to a partial hexon gene from

[12] Mag-Bind® FFPE DNA Kit, Omega Bio-Tek, Norcross, GA, USA.

Table 1
List of reported adenoviruses found in psittacine hosts.

Genus	Virus Isolate Name (Exemplar)	Abbreviated Name	Host Species	Documented Sequence Length	Corresponding Gene	Species (ICTV taxonomy)	GenBank Accession No.	Original Source
Atadenovirus	Psittacine adenovirus 3 isolate HKU/Parrot19	PsAdV-3	*Amazona farinosa*	Complete Genome	All	Psittacine atadenovirus A	KJ675568	To et al., 2014
Aviadenovirus	Poicephalus adenovirus isolate CS15-4016	PsAdV-4	*Poicephalus rufiventris*	Complete Genome	All	Psittacine aviadenovirus B	KX577802	Das et al., 2017
Aviadenovirus	Psittacine adenovirus 1 isolate GB 818-3	PsAdV-1	*Psittcula alexandri abbotti*	Partial cds, 1125 nt	Hexon	Not approved[A]	EF442329	Lüschow et al., 2007
Aviadenovirus	Psittacine adenovirus 1 isolate 18VIR149_ITA_2018	PsAdV-1	*Poicephalus senegalus*	Complete Genome	All	Not recognized	MH580295	Milani et al., 2018; Unpublished
Siadenovirus	Budgerigar adenovirus 1	BuAdV-1	*Melopsittacus undulatus*	Partial cds, 528-nt	Hexon	Not approved[A]	AB485763	Katoh et al., 2009
Siadenovirus	Psittacine adenovirus 2 isolate M114 clone 3	PsAdV-2	*Platycercus eximius*	Partial cds, 430-nt	Hexon	Not approved[A]	KC248185	Ballmann and Vidovszky, 2013
Siadenovirus	Plum headed parakeet adenovirus 1	PsAdV-2	*Psittacula cyanocephala & Cacatua alba*	Partial cds, 269-nt	DNA-dep DNA pol	Not approved[A]	EU056825	Wellehan et al., 2009
Siadenovirus	Psittacine adenovirus 2 isolate 7 & M115 clone 2, 3	PsAdV-2	*Cyanoramphus novaezelandiae, Melopsittacus undulatus, Neophema splendida, Nymphicus hollandicus, Platycercus eximius*	Partial cds, 269-nt	DNA-dep DNA pol	Not approved[A]	KC248192 KC248191 KC248192	Ballmann and Vidovszky, 2013
Siadenovirus	Psittacine adenovirus 2 isolate M78	PsAdV-2	*Melopsittacus undulatus*	Partial cds, 430-nt	Hexon	Not approvedA	KC248186	Ballmann & Vidovszky, 2013

[A] incomplete genome.

budgerigar adenovirus 1 (AB485763) (Katoh et al., 2009). The hexon gene encodes the major viral capsid protein and has been used for the phylogenetic study of adenoviruses (Davison et al., 2000; Kovács et al., 2003). The phylogenetic analysis based on the amino acid sequences of hexon proteins showed that this cockatiel virus was most closely related to the budgerigar adenovirus 1. The partial hexon gene from a scarlet chested parrot classified as PsAdV-2 (KC248185) does not overlap with budgerigar adenovirus 1 (AB485763); therefore, PsAdV-2 was excluded from the phylogenetic tree. Separately we were able to compare PsAdV-5 with PsAdV-2. BLASTn analysis indicates that PsAdV-5 isolate IA17-frag has 75% nucleotide identity with PsAdV-2 (KC248185).

There are two other reports that describe a siadenovirus, PsAdV-2, infecting seven different psittacine species. The reported sequences from PsAdV-2 include identical short sequences of the DNA pol gene from an umbrella cockatoo, a plum-headed parakeet (Wellehan et al., 2009), an eastern rosella, a scarlet chested parrot, a cockatiel, three budgerigars and a red-crowned parakeet (Ballmann and Vidovszky, 2013). Since several of the published PsAdV-2 sequences were of the DNA pol gene, we extracted DNA from formalin-fixed paraffin-embedded tissue in order to sequence this gene. We were able to amplify and sequence a portion of the DNA pol gene for comparison. Due to sequencing of both the inner and outer nested PCR products, we obtained a 441-nt consensus sequence that overlapped with the 269-nt sequences reported by Wellehan et al (2009) and Ballmann, et al (2013). We found that there is notable variation between the overlapping segments of PsAdV-5 and PsAdV-2 (only 70% nucleotide identity).

The species demarcation criteria for the genus *Siadenovirus* depends on differences in the host range and a phylogenetic distance of greater than 5–15% based on a distance matrix analysis of the DNA polymerase amino acid sequence (Harrach et al., 2011). A comprehensive phylogenetic analysis is difficult in this case since PsAdV-5 and PsAdV-2 are short partial DNA pol sequences; however, there does appear to be enough difference between both the hexon and DNA pol genes to suggest these viruses are different.

This manuscript describes a novel siadenovirus in a cockatiel. The hexon and partial DNA pol genes isolated from the cockatiel in this report are different from reported PsAdV-2 sequences. Instead the present isolate has a high degree of identity (99%) to budgerigar adenovirus 1, indicating that they likely represent the same virus. We propose that these isolates are representative of a distinct siadenovirus, psittacine adenovirus 5 (PsAdV-5). Herein we have documented the observation that PsAdV-5 is capable of crossing the species barrier, and it can infect at least two psittacine species (budgerigars and cockatiels). This is similar to PsAdV-2 which is known to infect at least seven different psittacine species. It is noteworthy that both of these siadenoviruses can readily infect multiple species of psittacine birds.

Disclosures

The authors have no conflicts of interest to declare. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Ballmann, M.Z., Harrach, B., 2016. Detection and partial genetic characterisation of novel avi- and siadenoviruses in racing and fancy pigeons (*Columba livia domestica*). Acta Vet. Hung. 64, 514–528. https://doi.org/10.1556/004.2016.047.

Ballmann, M., Vidovszky, M., 2013. Detection of broad-hostrange psittacine adenovirus (PsAdV-2) in representatives of different parrot species. Magy. Allatorvosok Lapja 135, 78–84.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Chen, Q., Wang, L., Zheng, Y., Zhang, J., Guo, B., Yoon, K.-J., Gauger, P.C., Harmon, K.M., Main, R.G., Li, G., 2018. Metagenomic analysis of the RNA fraction of the fecal virome indicates high diversity in pigs infected by porcine endemic diarrhea virus in the United States. Virol. J. 15, 95. https://doi.org/10.1186/s12985-018-1001-z.

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

Davison, A.J., Wright, K.M., Harrach, B., 2000. DNA sequence of frog adenovirus. J. Gen. Virol. 81, 2431–2439. https://doi.org/10.1099/0022-1317-81-10-2431.

Dhama, K., Gowthaman, V., Karthik, K., Tiwari, R., Sachan, S., Kumar, M.A., Palanivelu, M., Malik, Y.S., Singh, R.K., Munir, M., 2017. Haemorrhagic enteritis of turkeys – current knowledge. Vet. Q. 37, 31–42. https://doi.org/10.1080/01652176.2016.1277281.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evol. Int. J. Org. Evol. 39, 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x.

Fitzgerald, S.D., Reed, W.M., 1989. A review of marble spleen disease of ring-necked pheasants. J. Wildl. Dis. 25, 455–461. https://doi.org/10.7589/0090-3558-25.4.455.

Harrach, B., Benko, M., Both, G., Brown, M., Davison, A., Echavarría, M., Hess, M., Jones, M., Kajon, A., Lehmkuhl, H., Mautner, V., Mittal, S., Wadell, G., 2011. Family *Adenoviridae*. In: King, A., Adams, M., Carstens, E., Lefkowitz, E. (Eds.), Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, San Diego, pp. 125–141.

Joseph, H.M., Ballmann, M.Z., Garner, M.M., Hanley, C.S., Berlinski, R., Erdélyi, K., Childress, A.L., Fish, S.S., Harrach, B., Wellehan, J.F.X., 2014. A novel siadenovirus detected in the kidneys and liver of Gouldian finches (*Erythura gouldiae*). Vet. Microbiol. 172, 35–43. https://doi.org/10.1016/j.vetmic.2014.04.006.

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

Kovács, E.R., Benkő, M., 2009. Confirmation of a novel siadenovirus species detected in raptors: Partial sequence and phylogenetic analysis. Virus Res. 140, 64–70. https://doi.org/10.1016/j.virusres.2008.11.005.

Kovács, G.M., LaPatra, S.E., D'Halluin, J.C., Benko, M., 2003. Phylogenetic analysis of the hexon and protease genes of a fish adenovirus isolated from white sturgeon (Acipenser transmontanus) supports the proposal for a new adenovirus genus. Virus Res. 98, 27–34.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

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

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T.L., Agarwala, R., Schäffer, A.A., 2008. Database indexing for production MegaBLAST searches. Bioinforma. Oxf. Engl. 24, 1757–1764. https://doi.org/10.1093/bioinformatics/btn322.

Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, Oxford ; New York.

Park, Y.M., Kim, J.-H., Gu, S.H., Lee, S.Y., Lee, M.-G., Kang, Y.K., Kang, S.-H., Kim, H.J., Song, J.-W., 2012. Full genome analysis of a novel adenovirus from the South Polar skua (*Catharacta maccormicki*) in Antarctica. Virology 422, 144–150. https://doi.org/10.1016/j.virol.2011.10.008.

Raue, R., Gerlach, H., Müller, H., 2005. 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, 1933–1943. https://doi.org/10.1007/s00705-005-0578-x.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

To, K.K.W., Tse, H., Chan, W.-M., Choi, G.K.Y., Zhang, A.J.X., Sridhar, S., Wong, S.C.Y., Chan, J.F.W., Chan, A.S.F., Woo, P.C.Y., Lau, S.K.P., Lo, J.Y.C., Chan, K.-H., Cheng, V.C.C., Yuen, K.-Y., 2014. 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, e3318. https://doi.org/10.1371/journal.pntd.0003318.

Wellehan, J.F.X., Johnson, A.J., Harrach, B., Benkö, M., Pessier, A.P., Johnson, C.M., Garner, M.M., Childress, A., Jacobson, E.R., 2004. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. J. Virol. 78, 13366–13369. https://doi.org/10.1128/JVI.78.23.13366-13369.2004.

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

Wood, D.E., Salzberg, S.L., 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol. 15, R46. https://doi.org/10.1186/gb-2014-15-3-r46.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. J. Comput. Mol. Cell Biol. 7, 203–214. https://doi.org/10.1089/10665270050081478.

Zhang, J., Zheng, Y., Xia, X.-Q., Chen, Q., Bade, S.A., Yoon, K.-J., Harmon, K.M., Gauger, P.C., Main, R.G., Li, G., 2017. High-throughput whole genome sequencing of Porcine reproductive and respiratory syndrome virus from cell culture materials and clinical specimens using next-generation sequencing technology. J. Vet. Diagn. Investig. Off. Publ. Am. Assoc. Vet. Lab. Diagn. Inc 29, 41–50. https://doi.org/10.1177/1040638716673404.

Zsivanovits, P., Monks, D.J., Forbes, N.A., Ursu, K., Raue, R., Benko, M., 2006. Presumptive identification of a novel adenovirus in a Harris hawk (*Parabuteo unicinctus*), a Bengal eagle owl (*Bubo bengalensis*), and a Verreaux's eagle owl (*Bubo lacteus*). J. Avian Med. Surg. 20, 105–112.