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NOVEL ADENOVIRUS DETECTED IN KOWARI (*DASYUROIDES BYRNEI*) WITH PNEUMONIA

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A male kowari (*Dasyuroides byrnei*) originating from a zoo facility was delivered for post mortem evaluation in Hungary. Acute lobar pneumonia with histopathologic changes resembling an adenovirus (AdV) infection was detected by light microscopic examination. The presence of an AdV was confirmed by obtaining partial sequence data from the adenoviral DNA-dependent DNA-polymerase. Although the exact taxonomic position of this novel marsupial origin virus could not be determined, pairwise identity analyses and phylogenetic calculations revealed that it is distantly related to other members in the family *Adenoviridae*.

Keywords: Kowari adenovirus, brush-tailed marsupial rat, *Dasyuroides byrnei*, pneumonia

Introduction

Adenoviruses (AdVs) are frequently detected double-stranded DNA viruses of vertebrates (mammals, birds, squamata, chelonians, amphibians, and fish) usually exhibiting a narrow host-range and co-evolution with their host [1, 2]. AdV infections can be asymptomatic or associated with different clinical manifestations including conjunctivitis, respiratory illness, gastroenteritis, cystitis, meningoencephalitis, hepatitis, or even mortality. In most cases, the outcome of the infection is substantially influenced by the actual immune status of the host.

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Basophilic or eosinophilic nuclear inclusions in the affected susceptible cells are characteristic to AdV infections.

The family *Adenoviridae* is currently divided into five genera: *Mastadeno-*, *Aviadeno-*, *Atadeno-*, *Siadeno-*, and *Ichtadenovirus* (International Committee on Taxonomy of Viruses; http://www.ictvonline.org/). A sixth AdV lineage was described recently in testudinoid turtles in Hungary and USA [3, 4]. Although several AdVs have been identified in different mammalian species belonging to the infraclass Placentalia, there is only a single known marsupial origin AdV, the Possum AdV 1, which was described in brushtail possums (*Trichosurus vulpecula*, order Diprotodontia) from New Zealand [5]. Partial sequence data, high A+T content of the determined sequences and phylogenetic calculations clustered the Possum AdV 1 into the genus *Atadenovirus*.

The kowari or brush-tailed marsupial rat (*Dasyuroides byrnei*) is a small, 14–18 cm long nocturnal carnivorous marsupial native to the region of Lake Eyre in Australia. The kowari belongs to a different order in the infraclass Marsupialia, the Dasyuromorphia. Its diet consists of insects, lizards, rodents, birds, and eggs. There is limited information found in the literature about viral infections diagnosed in this species. Cytomegalic disease was described in several marsupial species including the kowari; historically, the disease was thought to be caused by a cytomegalovirus, but in a recent study gammaherpesviruses were detected in connection with the disorder [6]. The present work describes the detection, and preliminary genetic characterization of a distinct, novel AdV in a specimen of kowari as possible cause of pneumonia.

Pathological and Histological Investigations

On January 10, 2014, a succumbed male kowari (*D. byrnei*) was delivered to University of Veterinary Science, Department of Exotic Animal and Wildlife Medicine for post mortem evaluation. The kowari originated from a zoo facility in Budapest (Hungary). Routine pathological dissection was carried out with macroscopic examination. From organs showing visible pathological lesions (lung, liver, and kidneys), samples were taken for further histopathological, virological, and routine bacteriological investigations. Specimens intended for histopathological examination were fixed in 8% buffered formaldehyde solution and embedded in paraffin. Sections (4–5 μm thick) were stained with hematoxylin and eosin according to the standard histopathological procedure. Routine bacteriological examination from the organ samples was carried out on blood and Drigalski agar plates; cultures were incubated at 37 °C.

The necropsy performed on the carcass revealed normal fur and skin, without any changes in the natural orifices. The subcutis was intact with medium amount of subcutaneous fat tissue, indicating normal nutritional status of the kowari. Upon opening the abdominal cavity, the organs were located according to anatomical expectations. The spleen had normal shape and size, and was light brownish-red in color. The slightly enlarged liver was normally shaped, but yellowish and moderately easy to tear. The cut surface of the liver had a fatty shine. Neither the gastrointestinal tract nor the kidneys showed any pathological alterations. The position of the thoracic organs was normal, but the thoracic cavity contained 3 ml transparent thin fluid, the pleura visceralis was edematous (Figure 1). The lungs, in general, were normally shaped and sized, but the left caudal and the right middle lobes were enlarged. Most of the lung tissue appeared in the normal pale brick-red color, except for the enlarged lobes, which were mottled with darker red areas. The altered lobes had higher liquid content and contained less air bubbles. Although the heart was rounded, the myocardium and the chambers did not show any pathological lesions.

Histological examination of the liver revealed bile stagnation indicated by deposition of a homogenous brownish-red material in the capillaries, and

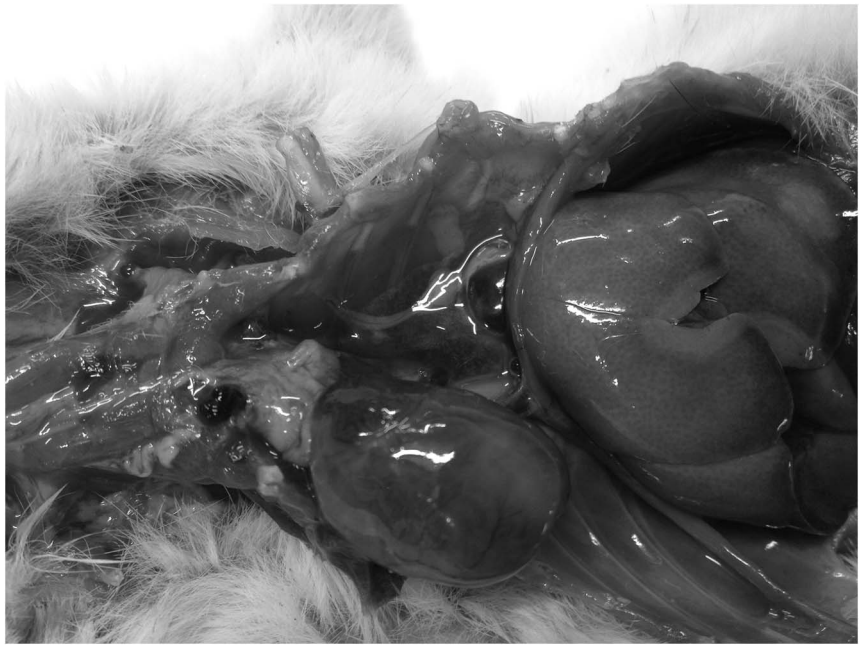

Figure 1. Fluid accumulation (hydrothorax) in the thoracic cavity of the kowari (*Dasyuroides byrnei*)

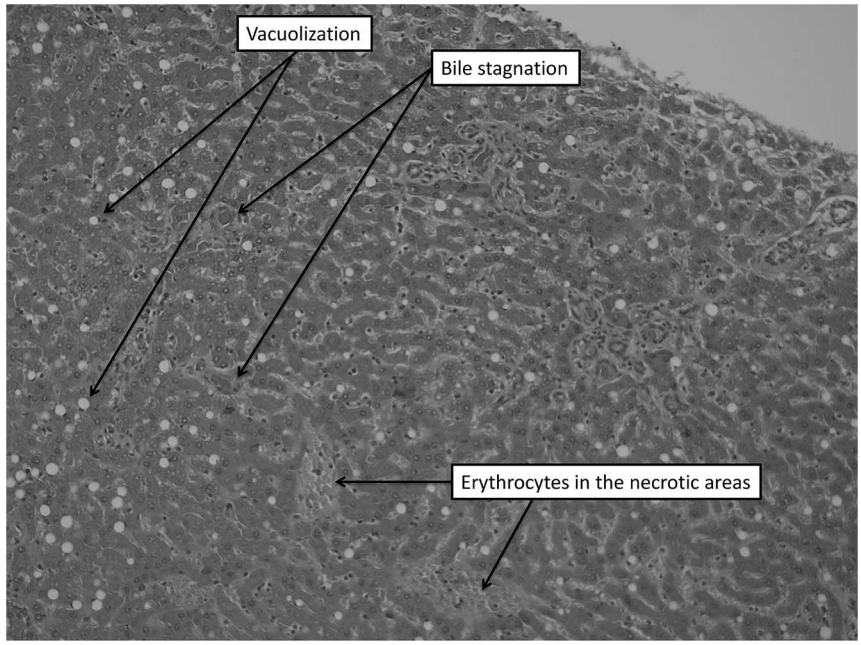

Figure 2. Liver dystrophy with signs of bile stagnation

hyperemia and dilation of the sinusoids. Multiple focal vacuolization and necrosis of the hepatocytes was also observed (Figure 2). The affected lung lobes showed pronounced hyperemia and inflammatory cell infiltration; thickening of the alveolar septa was observed. The lumen of the bronchioli contained groups of desquamated epithelial cells. In some areas, hyperplasia of the epithelium was also diagnosed (Figure 3). The pulmonary lesions were accompanied with mononuclear cell infiltration, giant cell formation, and intranuclear inclusion bodies in the alveolar epithelial cells (Figure 4). In the kidneys, multifocal subacute interstitial nephritis was seen along with tubulonephrosis which was characterized by vacuolization and necrosis of the tubular epithelial cells (Figure 5).

The routine bacteriological examination of the liver and the lung was negative; no pathogenic bacteria could be isolated.

Virological Investigations

Microscopic changes developed in almost every major organ, suggesting that the animal experienced viremia. Giant cell formation in the lung appears to be a fairly general viral phenomenon, not peculiar to any virus [7]; inclusion

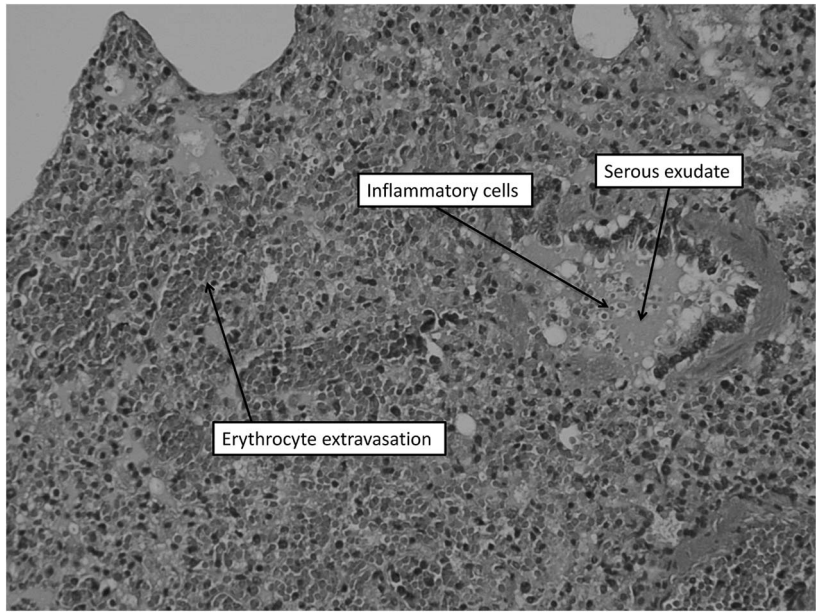

Figure 3. Hyperemia in the lung and desquamation of the epithelial cells in the airways

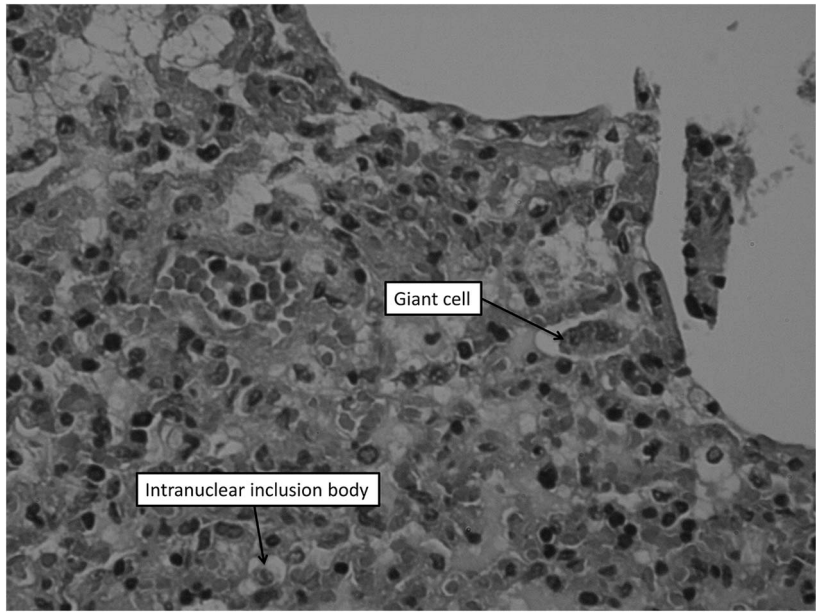

Figure 4. Giant cell formation, mononuclear inflammatory cell infiltration, and anisokaryosis in the lung

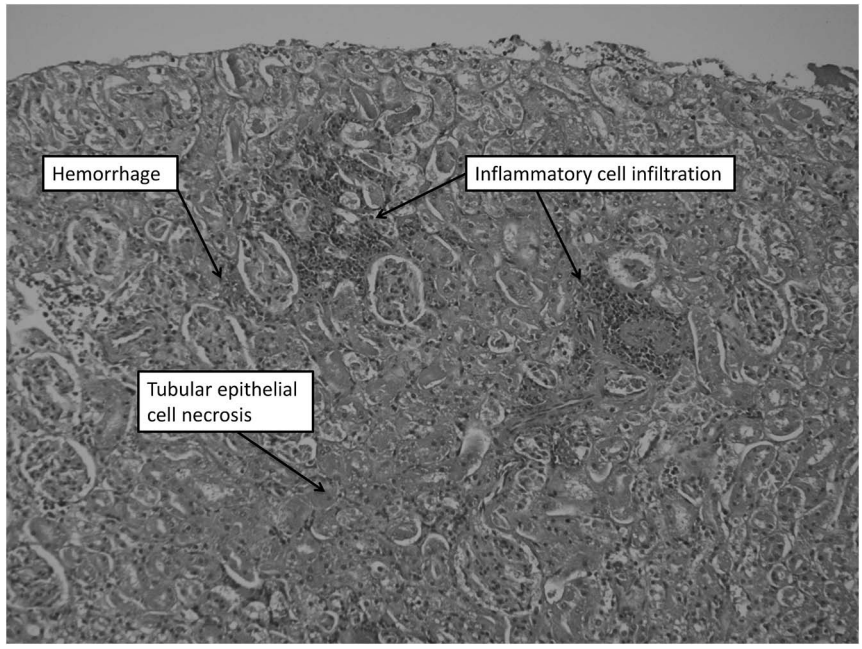

Figure 5. Tubulonephrosis and multifocal interstitial nephritis

bodies are characteristic to AdV infections, therefore AdV-specific nested PCR targeting the DNA-dependent DNA-polymerase gene was performed as described previously [8]. To exclude herpes-, circo-, and reovirus infections, consensus PCR assays, routinely used in our laboratory, were performed [9–11]. PCR products of the expected size were excised and then extracted from gel for direct sequencing. BLASTn and BLASTx algorithms were used to identify homologous genes among sequences deposited in GenBank [12]. AdV sequences used in our study were retrieved from GenBank. Multiple sequence alignments were generated using the Multalin online platform (http://multalin.toulouse.inra.fr/multalin/) and sequences were edited in GeneDoc [13]. Phylogenetic analysis was performed using the MEGA version 6.0. program package [14]. Gene-specific substitution models were evaluated, and the best-fit model was selected based on the Bayesian information criterion. A maximum likelihood tree was generated, and tree topology was validated by bootstrap analysis as implemented in MEGA6. Pairwise identity comparison based on aligned amino acid (aa) sequences was also calculated in MEGA6 (p-distance, uniform rates, and pairwise deletion).

Table I. Pairwise amino acid sequence identity values calculated between members of a certain genus, and the Kowari adenovirus and members of four established genera (*Atadeno-*, *Siadeno-*, *Aviadeno-*, and *Mastadenovirus*) and members of a new genus with the proposed name *Testadenovirus* in the family *Adenoviridae*

	Kowari adenovirus	*Atadenovirus*	*Siadenovirus*	*Aviadenovirus*	*Mastadenovirus*	*Testadenovirus*
Kowari adenovirus	**100%**	53.3%–58.9%	46.7%–51.7%	47.2%–51.1%	48.9%–61%	41.1%–46.7%
Atadenovirus		**54.4%–100%**	46.3%–58.9%	42.2%–57.8%	42.2%–59.0%	37.3%–48.9%
Siadenovirus			**52.8%–82.2%**	48.3%–53.9%	44.4%–55.6%	36.7%–41.6%
Aviadenovirus				**72%–93.9%**	46.7%–55.1%	41.1%–43.3%
Mastadenovirus					**60.1%–87.8%**	40.0%–48.9%
Testadenovirus						**69.2%–94.5%**

Note: Bold values indicate the identity values between members of a certain genus.

Nucleic acid samples isolated from pooled organ samples (lung, liver, and kidney) were used for the PCR-based detection of numerous pathogens, but only the AdV-specific PCR gave positive result. The sequence data was submitted to GenBank (accession no.: KT696557). The G+C content of the Kowari AdV sequence was relatively high, 65%, but fell in the range observed in other adenoviral

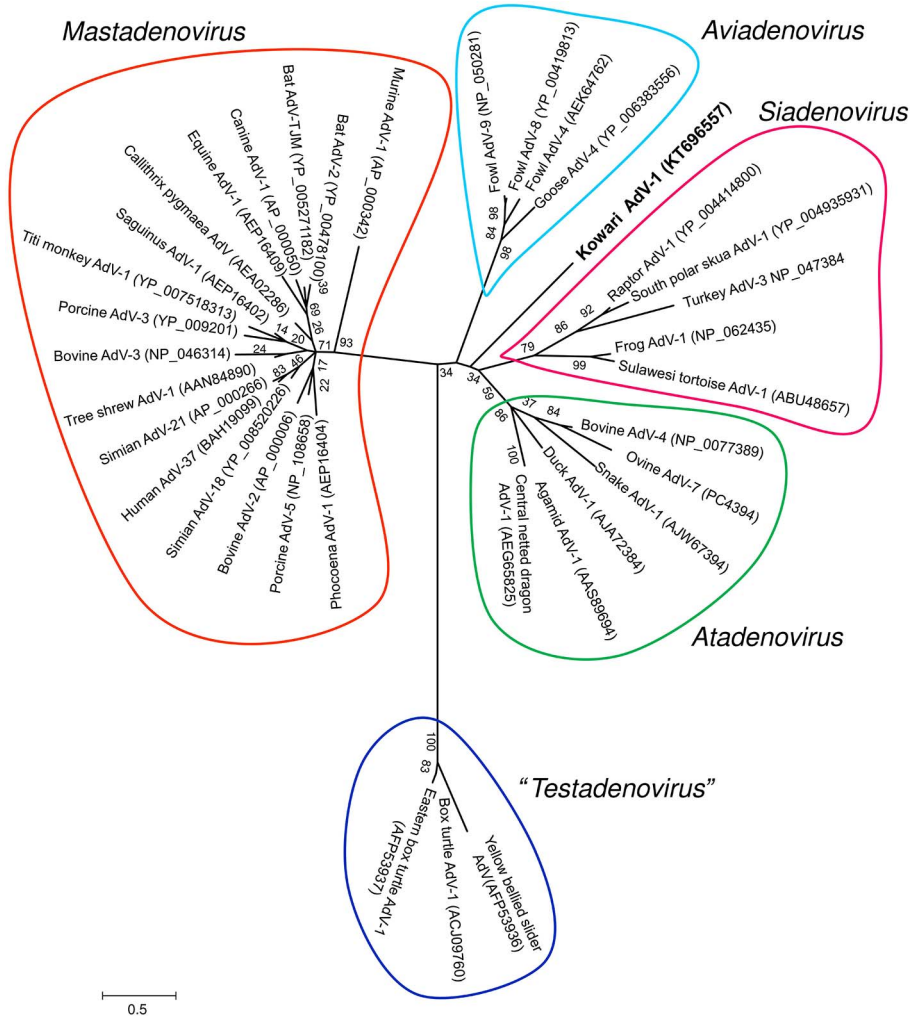

Figure 6. Unrooted phylogenetic tree of partial adenoviral DNA-dependent DNA-polymerase amino acid sequences showing the clustering of adenoviruses. The length of the branches indicates the phylogenetic distance between the different viruses and the scale bar represents 50 mutations per 100 sequence positions. Bootstrap values are given for 100 data sets

sequences (33.6%–66.9%). BLASTx analysis of the partial, 90 aa long sequence of the Kowari AdV revealed that the highest scores were obtained when compared with the Bearded dragon AdV 1 (GenBank accession no.: AAS89694) and Central netted dragon AdV 1 (GenBank accession no.: AEG65825). Amino acid sequence identity values between the Kowari AdV and the studied sequences ranged between 41.1% and 61% (Yellow-bellied slider AdV and Porcine AdV 3, GenBank accession no.: AGB07604 and AC_000189, respectively). Of interest, the observed aa sequence identity values along this short genomic region fell, virtually, in the range distinguishing established AdV genera (Table I). Although in the phylogenetic calculations, the Kowari AdV did not cluster with viruses belonging to established AdV genera and appeared on a separate branch, conclusions about the taxonomic position of this novel AdV cannot be drawn based on the short DNA-polymerase gene sequence (Figure 6). Unfortunately no overlapping sequences with Possum AdV 1 were available in GenBank for comparison, but our data suggests that there is no close genetic relationship between the two marsupial origin AdVs. Additional sequencing of genes reflecting more precisely the phylogenetic relationships of AdVs is needed in order to classify this novel virus.

Conclusions

The pathological and the virological examinations of the kowari's carcass (*D. byrnei*) revealed acute lobar pneumonia associated with an AdV infection, pronounced hydrothorax, liver dystrophy with bile stagnation, and subacute multifocal nephritis and tubulonephrosis. We assume that the direct cause of death of the animal might have been the respiratory failure and consequential hypoxemia of the parenchymal organs. The kowari was kept in a cage with a companion animal, which was supposedly infected by the same virus, but remained healthy. Therefore, it can be presumed that a possible immunocompromised state or unknown stress factors might have exacerbated the AdV infection in the animal, which could lead to viremia and the consequential death of the animal. Although the exact pathogenic role of the novel Kowari AdV strain has not been examined, the pronounced genetic distance of the detected virus from other known AdVs justifies the further investigations of AdV diversity and pathogenicity in marsupials.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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