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Short communication

Phylogenetic analysis of a novel reassortant orthoreovirus strain detected in partridge (*Perdix perdix*)

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ABSTRACT

Avian orthoreoviruses cause various diseases in wild birds and domesticated poultry. In this study we report the detection and genomic characterization of a partridge (*Perdix perdix*) origin reovirus strain, D1007/2008. The virus was isolated on cell culture from acute pneumonia and infra-orbital sinusitis. The 23,497 nucleotide long genome sequence was obtained by combined use of semiconductor and capillary sequencing. Sequence and phylogenetic analyses showed that the partridge reovirus strain was related to orthoreoviruses of gallinaceous birds. In fact, five (λB, λC, μB, σC, σNS) and one (σB) out of 10 genes clustered definitely with turkey or chicken origin orthoreoviruses, respectively, whereas in the λA, μA, μNS and σA phylogenies a more distant genetic relationship was observed. Our data indicate that the identified reovirus strain is composed of a mixture of chicken and turkey orthoreovirus related alleles. This finding implies that partridges may serve as natural reservoirs for orthoreoviruses of domesticated poultry.

Orthoreoviruses are characterized by medium sized (70–80 nm) icosahedral virion, composed of multiple concentric protein layers with protruding turrets at the 5-fold axes. The reovirus (RV) genome is 10-segmented double-stranded RNA and is classified into three size classes, such as L (large, containing L1–L3 segments), M (medium, M1–M3) and S (small, S1–S4). Genomic segments are monocistronic, except for the S1 or the S4 segment, which encodes two or three partially overlapping ORFs in a strain-dependent manner. The full coding capacity is 11 or 12 ORFs (Benavente and Martínez-Costas, 2007).

The genus *Orthoreovirus* (family *Reoviridae*) officially contains five virus species (Attoui et al., 2011). Orthoreoviruses of birds are classified into the species *Avian orthoreovirus* and into tentative species, such as Tvärminne avian orthoreovirus and Bulbul orthoreovirus (Dandár et al., 2014; Ogasawara et al., 2015). The species *A. orthoreovirus* includes all currently known reovirus strains from chicken, turkey, and waterfowl species. The occurrence of reoviruses has been documented in additional avian species, although in most cases the genetic features of these strains detected in wild birds and exotic zoo and pet birds are unexplored.

Avian reoviruses cause various clinical manifestations in their respective host species. The outcome of infection depends on numerous factors, including the age, breed and immune status of the affected birds. The clinical picture becomes complicated when multiple pathogens co-infect an animal. In chicken, the classical form of reovirus disease is tenosynovitis. Additionally, runting-stunting syndrome, hepatitis, myocarditis, and central nervous system infections have been reported to be caused by reoviruses in chickens (Bányai et al., 2011; Dandár et al., 2013; Davis et al., 2012; Troxler et al., 2013). In turkey, tenosynovitis, myocarditis and poult enteritis and mortality syndrome have been associated with reovirus infection (Heggen-Peay et al., 2002; Sharafeldin et al., 2014; Shivaprasad et al., 2009). In waterfowl, particularly in Muscovy ducks, two major diseases have been described. One disease form occurs in young ducklings, manifests in lethargy, weakness, diarrhea, lameness, respiratory signs with tenosynovitis, pericarditis, enlargement and small necrotic foci in the spleen and the liver (Farkas et al., 2014). The other form causes significantly higher mortality (up to 60%) not only in Muscovy but also in Pekin duck-

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Table 1
General features of the genome of partridge reovirus strain D1007/2008.

Genome segment	Size (nt)	Length of the			Sequence at the termini (5′ end/. . ./3′ end)	Encoded protein	Protein size (aa)
		5′ end	ORF	3′end			
L1	3958	20	3882	56	GCUUUUC/.../UCAUC	λA (Core shell)	1293
L2	3907	12	3858	37	GCUUUUC/.../UCAUC	λC (Core turret)	1285
L3	3830	14	3780	36	GCUUUUU/.../UCAUC	λB (Core RdRp)	1259
M1	2284	13	2199	72	GCUUUUU/.../UCAUC	μA (Core NTPase)	732
M2	2158	29	2031	98	GCUUUUU/. . ./UCAUC	μB (Outer shell)	676
M3	1996	24	1908	64	GCUUUUU/. . ./UCAUC	μNS (NS factory)	635
S1	1646	24	306453981	33	GCUUUUU/. . ./UCAUC	p10 (NS FAST) p17 (NS other) σC (Outer fiber)	101 150 326
S2	1324	15	1251	58	GCUUUUU/. . ./UCAUC	σA (Core clamp)	416
S3	1202	30	1104	68	GCUUUUU/. . ./UCAUC	σB (Outer clamp)	367
S4	1192	23	1104	65	GCUUUUU/. . ./UCAUC	σNS (NS RNAb)	367

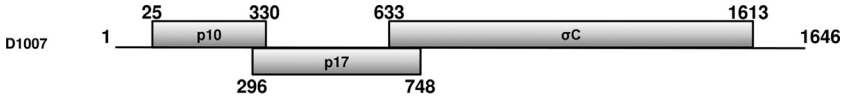

Fig. 1. Gene arrangement of the S1 genome segment of the partridge reovirus strain D1007/2008.

Table 2
Gene-specific nucleotide (nt) and amino acid (aa) sequence identities of the partridge reovirus strain D1007/2008 to other avian origin reoviruses.

Segment	Encoded protein	D1007/2008									
		Chicken RV		Turkey RV		Waterfowl RV		Crow RV		Bulbul RV	
		nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
L1	λA	78.9–79.5	95.4–95.8	78.3–78.6	95.1–95.4	76.9–77.9	93.5–94.7	71.7	81.4	69	78.6
L2	λC	72.3–86.7	82.1–92.3	88.3–89.3	93.9–94.5	69.3–70.3	77.9–78.1	46.5	38.1	46.9	38.6
L3	λB	82.8–84.7	94.9–96.3	91.3–92.8	97.1–97.9	75.4–76.1	90.7–91.7	63.3	70.8	64.6	72
M1	μA	79–87.3	91.7–95.4	79.1–79.7	90.7–91.7	72.4–74.5	83.6–85.2	55.7	56.6	57.5	55.7
M2	μB	74–79.2	88–92.7	74.7–77.7	88.4–93.5	68.5–77.3	76.7–93.3	67	74.2	68.3	77.6
M3	μNS	80.1–85.6	90.1–94.5	81–81.1	89.1–89.6	70.8–71.3	79.2–80.5	50.2	44	48.1	38.4
S1	σC	56.8–60.1	51.5–55.2	84.3–85.4	81.8–83.7	40–41.1	28.1–29.3	43.6	34.8	52.5	45.1
S2	σA	81.9–87	96.6–98.3	80.3–82.1	95.9–96.9	75.9–77.1	89.9–91.6	62.3	65.1	59.2	58.7
S3	σB	82.6–84.9	87.9–90.1	67.4–69.3	75.1–77.9	60–64.7	60–66.3	47.5	38.4	49.3	40.2
S4	σNS	80.3–81.5	92.6–93.2	83.9–85.1	93.6–95.1	78.8–79.9	90.5–91.8	59.7	56.8	55.3	55.9

lings and goslings; the syndrome has been described in China and referred to as spleen necrosis disease referring to the marked haemorrhagic-necrotic pathologic lesions in the spleen (Yun et al., 2014). In Hungarian geese, Palya et al. (2003) have described splenitis and hepatitis during the acute phase, and epicarditis, arthritis and tenosynovitis during the subacute and chronic phase. Amongst wild birds reovirus infection was associated with diarrhea in pheasant (*Phasianus colchicus*) (Mutlu et al., 1998), wasting in American woodcock (*Scolopax minor*) (Docherty et al., 1994), gastroenteritis in Virginia quails (*Colinus virginianus*) (Ritter et al., 1986), and neurological signs in hooded crow (*Corvus corone cornix*) (Huhtamo et al., 2007).

The role of wild birds in the maintenance and transmission of reoviruses has been hypothesized for a while. The σA gene of an avian reovirus strain isolated from healthy ostrich (*Struthio camelus*) raised on a breeder farm in Japan showed great sequence similarity to chicken origin reoviruses (Sakai et al., 2009). More recently, another reovirus strain detected in a free-living magpie was found to be genetically related to chicken origin reoviruses based on the σC encoding gene phylogeny (Lawson et al., 2015). The apparent genetic relationship in partial genome sequences between reoviruses of wild or exotic birds and those detected in commercial poultry seems to reinforce the hypothesis on the possible reservoir role of feral birds in reovirus transmission. Available genetic information, however, shows some limitations regarding the interpretability of data in the absence of complete gene configurations of the strains detected in species other than commercial poultry.

This study reports the detection, isolation and genomic characterization of a novel reovirus strain from partridge (*Perdix perdix*). As no reports have indicated so far that partridge hosts reovirus, understanding the genetic features of this particular strain seemed to be of interest.

A partridge breeding farm managed by local hunter society in central Hungary reported sporadic daily deaths and clinical signs of infra-orbital sinusitis in approximately 5–6% of young birds during early autumn of 2008. Pathological/histopathological examination revealed pneumonia in nine randomly selected dead animals. As a specific request, detection of adenovirus infection was ordered from the diagnostic laboratory. Accordingly, LMH cells (primary chicken hepatocellular carcinoma epithelial cell line, ATCC® CRL-2117™) were inoculated with antibiotics treated suspensions of organ samples from dead animals. As a result, giant cell formation, the cytopathic effect typical to avian reovirus infection appeared shortly after inoculation. Reverse transcription-PCR and sequencing of the σNS gene amplified from the cell culture supernatant identified a reovirus strain, designated D1007/2008. Evidence of adenovirus infection was not uncovered.

To further characterize the isolated reovirus strain, it was subjected to whole genome sequencing using the protocol described in detail elsewhere (Bányai et al., 2013). In brief, RNA was extracted using TRIzol Reagent (Sigma–Aldrich, Saint Louis, MO, USA) accord-