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Novel reovirus isolation from an Ostrich (*Struthio camelus*) in Japan

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ABSTRACT

An orthoreovirus was isolated from an Ostrich (*Struthio camelus*) and rapidly identified as orthoreovirus by the rapid determination of viral RNA sequences (RDV) system and electron microscopy. Phylogenetic analysis of the sigma A protein indicated that the isolate belonged to avian species and was closely related to chicken orthoreovirus strain 138. The results of the present study indicated that an ostrich orthoreovirus is slight different from other chicken orthoreoviruses and provided evidence of diversity among avian orthoreoviruses. To our knowledge, this is the first genetic report of an orthoreovirus isolated from an ostrich.

1. Introduction

Commercial ostrich farms were introduced in Japan in 1988, and the number of ostriches increased rapidly over the next 15 years, exceeding 9000 birds by 2002. As ostriches are kept mainly under free-range conditions, they can easily come into contact with wild birds and animals that may be infected with pathogens, including bacteria, viruses and parasites. In our previous serological surveillance of Newcastle disease virus (NDV) in Japanese ostriches, 12.2% of 181 slaughter-age ostriches were shown to contain NDV-specific neutralizing antibodies without any vaccination history (Sakai et al., 2006). Also, NDV was also isolated from wild birds that may have come into contact with these ostriches (Sakai et al., 2007b). These observations indicated that NDV has infiltrated into ostrich farms in Japan. However, little information is available regarding infectious diseases other than Newcastle disease and avian influenza in ostriches. As the first step to identify unidentified viruses in ostriches, we attempted to isolate viruses and to accumulate information regarding viral nucleic acid sequences using a rapid determination of viral RNA sequences (RDV) system that has been used successfully for identification of RNA viruses (Mizutani et al., 2007; Sakai et al., 2007a; Kihara et al., 2007; Maeda et al., 2008; Watanabe et al., 2008).

2. Materials and methods

2.1. Virus isolation and virological characterization

Fresh intestinal content samples of slaughter-age ostriches (12 months old, apparently healthy) were obtained from ostrich breeder farm in Yamagata prefecture in Japan and put into PBS containing antibiotics (penicillin 10,000 units/ml, streptomycin 10,000 μg/ml, gentamycin 5000 μg/ml, amphotericin B 50 μg/ml) to give a 20% suspension. The suspension was centrifuged at 12,000 × *g* for 5 min. Aliquots of 200 μl of the supernatant were inoculated into primary chicken kidney cells (CKC) according to the methods described previously (Kawamura et al., 1965).

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Table 1
Primers used for the rapid determination of viral RNA sequences system

Sequence code	HaeIII primer sequence (5′–3′)	AluI primer sequence (5′–3′)
Forward primer		
H1-1	AATTCGGCGGCCGCGGATCCCCGGGG	AATTCGGCGGCCGCGGATCCCTGGGG
Reverse primer		
H9-1	AATTCGGCGGCCGCGGATCCCCAGGG	AATTCGGCGGCCGCGGATCCCTAGGG
H9-2	AATTCGGCGGCCGCGGATCCCCAGGC	AATTCGGCGGCCGCGGATCCCTAGGC
H9-3	AATTCGGCGGCCGCGGATCCCCAGGA	AATTCGGCGGCCGCGGATCCCTAGGA
H9-4	AATTCGGCGGCCGCGGATCCCCAGGT	AATTCGGCGGCCGCGGATCCCTAGGT
H9-5	AATTCGGCGGCCGCGGATCCCCAGCG	AATTCGGCGGCCGCGGATCCCTAGCG
H9-6	AATTCGGCGGCCGCGGATCCCCAGCC	AATTCGGCGGCCGCGGATCCCTAGCC
H9-7	AATTCGGCGGCCGCGGATCCCCAGCA	AATTCGGCGGCCGCGGATCCCTAGCA
H9-8	AATTCGGCGGCCGCGGATCCCCAGCT	AATTCGGCGGCCGCGGATCCCTAGCT
H9-9	AATTCGGCGGCCGCGGATCCCCAGAG	AATTCGGCGGCCGCGGATCCCTAGAG
H9-10	AATTCGGCGGCCGCGGATCCCCAGAC	AATTCGGCGGCCGCGGATCCCTAGAC
H9-11	AATTCGGCGGCCGCGGATCCCCAGAA	AATTCGGCGGCCGCGGATCCCTAGAA
H9-12	AATTCGGCGGCCGCGGATCCCCAGAT	AATTCGGCGGCCGCGGATCCCTAGAT
H9-13	AATTCGGCGGCCGCGGATCCCCAGTG	AATTCGGCGGCCGCGGATCCCTAGTG
H9-14	AATTCGGCGGCCGCGGATCCCCAGTC	AATTCGGCGGCCGCGGATCCCTAGTC
H9-15	AATTCGGCGGCCGCGGATCCCCAGTA	AATTCGGCGGCCGCGGATCCCTAGTA
H9-16	AATTCGGCGGCCGCGGATCCCCAGTT	AATTCGGCGGCCGCGGATCCCTAGTT

2.2. Rapid determination of viral RNA sequences (RDV) system

To identify the isolated virus, the rapid determination of viral RNA sequences (RDV) system version 2.1 (Sakai et al., 2007a and unpublished data) was applied. Briefly, culture supernatant was treated with DNase I and RNase A, following RNA extraction by ISOGEN-LS (Nippon Gene, Tokyo, Japan). Aliquots of 10 μl of RNA solution were amplified using a whole transcriptome amplification

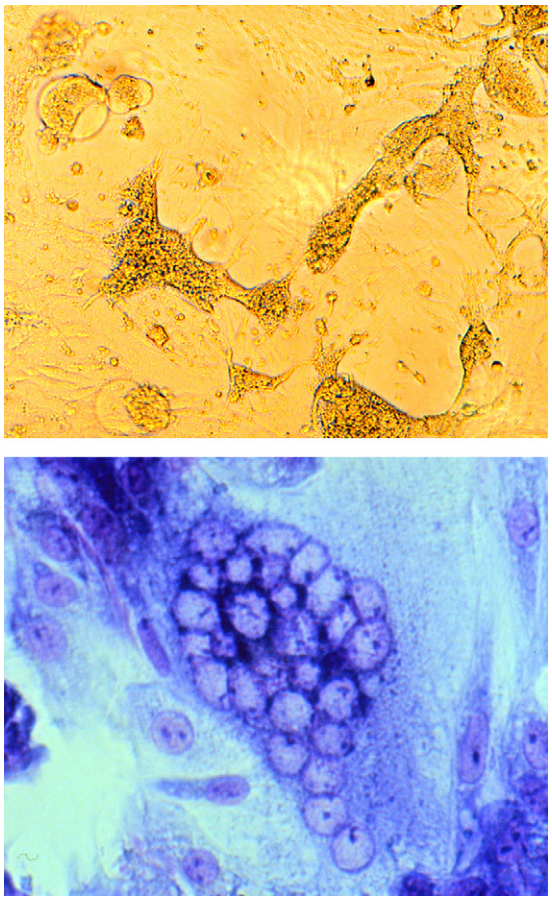

Fig. 1. Syncytia formation produced by infection of chicken kidney cells with Ost1 isolated from the ostrich.

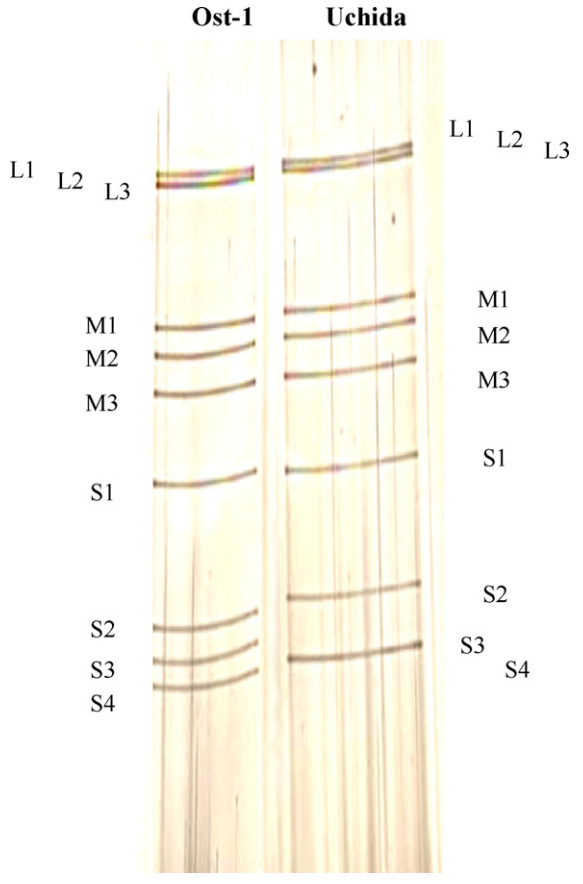

Fig. 2. Electrophoretic patterns of the genomic RNA of the Ost1 and avian orthoreovirus strain Uchida.