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Epizootic canine distemper virus infection among wild mammals

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ABSTRACT

In the spring of 2007, seven raccoon dogs and a weasel were captured near the city of Tanabe in Wakayama prefecture, Japan. The causative agent of the animals' death 1–2 days after capture was identified as canine distemper virus (CDV) by virus isolation, immunostaining with an anti-CDV polyclonal antibody, and a commercially available CDV antigen-detection kit. Sequence analysis of hemagglutinin genes indicated the isolated viruses belong to genotype Asia-1 and possess the substitution from tyrosine (Y) to histidine (H) at position 549 that is associated with the spread of CDV to non-canine hosts. A serosurvey for CDV was then conducted among wild animals in the region. The animals assayed consisted of 104 raccoons, 41 wild boars, 19 raccoon dogs, five Sika deer, two badgers, one weasel, one marten, one Siberian weasel and one fox. Virus-neutralization (VN) tests showed that, except for fox and weasel, all of the species assayed had VN antibodies to CDV. Interestingly, 11 of the 41 wild boars (27%) and two of the five Sika deer assayed possessed VN antibodies to CDV. These findings indicate that CDV infection was widespread among wild mammals during this epizootic.

1. Introduction

Canine distemper virus (CDV) is a non-segmented, negative-stranded, enveloped RNA virus of the order *Mononegavirale*, family *Paramyxoviridae* and genus *Morbillivirus*. CDV infects dogs and a variety of carnivore species, causing a fatal disease that manifests as pyrexia, anorexia, nasal discharge, conjunctivitis, diarrhea, leukopenia, and encephalitis (Appel, 1969; Appel and Summers, 1995). Vaccination with attenuated live virus is typically used to protect domestic dogs and cases of dog deaths by CDV are currently rare in Japan. However, CDV strains that are genetically distinct from the one used to produce the live vaccine (Onderstepoort strain), have recently been isolated in different regions of the world, and at least two of these strains, genotypes Asia-1 and Asia-2, have spread throughout Asia and Japan (Mochizuki et al., 1999).

Epizootics of CDV in wild animals are considered to be a serious global problem. For example, the famous discovery of a "mass grave" of lions (*Panthera leo*; Felidae) in the Serengeti National Park of Tanzania in 1994 was later attributed to CDV infection (Roelke-Parker et al., 1996). In California, the island fox (*Urocyon littoralis*) has become endangered due to CDV epizootics (United States Fish and Wildlife Service, 2004; Timm et al., 2009). In Japan, CDV infections have been reported in wild animals from a variety of regions (Machida et al., 1992, 1993; Ohashi et al., 2001; Hirama et al., 2004; Watabe and Yoshizawa, 2006; Yoshizawa and Watabe, 2007; Takayama et al., 2009). In 2008, CDV cases were reported in rhesus monkeys and crab-eating monkeys in China and Japan, respectively

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(Morikawa et al., 2008; Sun et al., 2010). This study reports the findings of a CDV serological survey that was conducted among wild animals after a CDV epizootic in the vicinity of Tanabe in Wakayama prefecture, Japan, in 2007.

2. Materials and methods

2.1. CDV epizootic

Several raccoon dog deaths were reported since the end of March, 2007 around Tanabe in Wakayama Prefecture, Japan. The raccoon dogs presented with prostration and an inability move, and died within 1–2 days after being sheltered. Raccoon dog No. 729 died on April 16, 2007, was sent to Yamaguchi University for diagnosis of the causative agent. A total of seven raccoon dogs and one weasel (No.734) were subsequently diagnosed as being infected with CDV. Although no further cases were reported after May 16, 2007, one raccoon dog (No.812) from the same area died due to CDV infection on January 2008. The characteristics of these infections are summarized in Table 1.

2.2. Cells

Vero cells (Japanese Collection of Research Bioresources (JCRB) Number: JCRB9013) were cultured in Eagle's minimum essential medium (EMEM; Gibco, USA) with 5% heat-inactivated fetal calf serum (FCS; Gibco), 1 mM sodium pyruvate, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO_2. Canine A72 cells (American Type Culture Collection (ATCC) Number: CRL-1542), feline CRFK cells (ATCC Number: CCL-94), and A72/cSLAM and CRFK/cSLAM cells expressing canine SLAM (Nakano et al., 2009a,b) were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% FCS and antibiotics at 37 °C in 5% CO_2.

2.3. Viruses

CDV KDK-1 (genotype Asia-1) (Mochizuki et al., 1999) strain was propagated in A72/cSLAM cells, and the Onderstepoort strain (vaccine) was propagated in Vero cells. W729B and W812B strains were isolated from the brains of raccoon dogs No.729 and No.812 in Wakayama Prefecture in 2007 and 2008, respectively, and then isolated and propagated in A72/cSLAM cells.

2.4. Virus isolation

DMEM containing high concentrations of antibiotics was added to swabs and tissues and then vortexed or homogenized. The samples were centrifuged and the supernatants were filtrated through a 0.45 μm filter. The filtrates or sera were then used to inoculate A72/cSLAM cells expressing canine SLAM. The inoculated cells were cultured until cytopathic effect was observed.

2.5. Serum samples

A total of 129 sera were collected from wild animals captured around Tanabe in Wakayama Prefecture from 2007 to 2008. Wild animals included 104 raccoons (*Procyon lotor*), 19 raccoon dogs (*Nyctereutes procyonoides*), two badgers (*Meles meles*), one weasel (*Mustela itatsi*), one Japanese marten (*Martes melampus*), one Siberian weasel (*Mustela sibirica*), one red fox (*Vulpes vulpes*), 41 wild boars (*Sus scrofa*) and five Sika deer (*Cervus nippon*). Furthermore, 28 and 20 sera were collected from wild boars and Sika deer, respectively, captured around Tanabe in Wakayama Prefecture from 2009 to 2010. All sera were heat-inactivated at 56 °C for 30 min and stored at −20 °C until use.

2.6. Virus neutralizing (VN) test

VN test for KDK-1 was performed by a 75% plaque-reduction neutralizing test ($PRNT_{75}$) using our established cell line, CRFK/cSLAM (Nakano et al., 2009a,b). For the first screening of CDV-positive sera, 10 μl of sera was added to 90 μl of virus solution containing approximately 100 plaque forming units (PFU) of KDK-1 diluted with DMEM supplemented with 2% FCS and incubated at 37 °C for 1 h (1:10 dilution). Then, 50 μl of the mixture was added to each well of a 24-well plate (Sumilon, Japan) subconfluented with CRFK/cSLAM. The plate was incubated at 37 °C for 1 h, washed twice with DMEM without FCS and overlaid with DMEM containing 0.8% agarose and 7% FCS. Plates were then incubated at 37 °C in 5% CO_2 for 3–4 days.

Table 1
Characteristics of virus isolates.

No.	Species	Date (yr/m/d)	Sex	Body weight (kg)	Virus isolate[a]	CDV antigen[b]
729	Raccoon dog	2007/4/16	M[c]	2.35	B, U	N.D.[d]
731	Raccoon dog	2007/4/20	F	2.80	F	+
734	Weasel	2007/4/25	M	0.50	B	+
739	Raccoon dog	2007/5/11	F	3.05	–	+
741	Raccoon dog	2007/5/15	F	3.00	F	+
742	Raccoon dog	2007/5/15	F	3.30	–	+
743	Raccoon dog	2007/5/15	F	2.25	U, F	+
744	Raccoon dog	2007/5/16	M	2.20	U, F	+
812	Raccoon dog	2008/1/31	F	2.35	B, U, F, S	+

[a] CDV were isolated from brain (B), fecal sample (F), urine (U) and/or serum (S).
[b] CDV antigen was detected by Checkman CDV and/or immunohistopathology using a CDV-specific polyclonal antibody.
[c] M, male and F, female.
[d] N.D.: not done.