FULL PAPER *Virology*

An Outbreak of Canine Distemper Virus in Tigers (*Panthera tigris*): Possible Transmission from Wild Animals to Zoo Animals

Yumiko NAGAO[1)], Yohei NISHIO[1)], Hiroshi SHIOMODA[1)], Seiji TAMARU[1)], Masayuki SHIMOJIMA[1)], Megumi GOTO[2)], Yumi UNE[3)], Azusa SATO[4)], Yusuke IKEBE[4)] and Ken MAEDA[1)]*

[1)]*Laboratory of Veterinary Microbiology, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan*
[2)]*Laboratory of System Physiology, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan*
[3)]*Laboratory of Veterinary Pathology, Azabu University, 1–17–71 Fuchinobe, Kanagawa 252–5201, Japan*
[4)]*Akiyoshidai Safari Land, 1212 Aka, Mito, Mine, Yamaguchi 754–0302, Japan*

(Received 10 November 2011/Accepted 16 December 2011/Published online in J-STAGE 28 December 2011)

ABSTRACT. Canine distemper virus (CDV), a morbillivirus that causes one of the most contagious and lethal viral diseases known in canids, has an expanding host range, including wild animals. Since December 2009, several dead or dying wild raccoon dogs (*Nyctereutes procyonoides*) were found in and around one safari-style zoo in Japan, and CDV was isolated from four of these animals. In the subsequent months (January to February 2010), 12 tigers (*Panthera tigris*) in the zoo developed respiratory and gastrointestinal diseases, and CDV RNA was detected in fecal samples of the examined tigers. In March 2010, one of the tigers developed a neurological disorder and died; CDV was isolated from the lung of this animal. Sequence analysis of the complete hemagglutinin (H) gene and the signal peptide region of the fusion (F) gene showed high homology among these isolates (99.8–100%), indicating that CDV might have been transmitted from raccoon dog to tiger. In addition, these isolates belonged to genotype Asia-1 and had lower homology (<90%) to the vaccine strain (Onderstepoort). Seropositivity of lions (*Panthera leo*) in the zoo and wild bears (*Ursus thibetanus*) captured around this area supported the theory that a CDV epidemic had occurred in many mammal species in and around the zoo. These results indicate a risk of CDV transmission among many animal species, including large felids and endangered species.
KEY WORDS: canine distemper, raccoon dog, tiger.

doi: 10.1292/jvms.11-0509; *J. Vet. Med. Sci.* 74(6): 699–705, 2012

Canine distemper virus (CDV) is an enveloped, negative-sense, single-stranded RNA virus [16] belonging to genus *Morbillivirus*, family *Paramyxoviridae*, order *Mononegavirales*, and causes lethal disease including pyrexia, anorexia, nasal discharge, diarrhea, lymphopenia, and encephalitis [1]. Traditionally, CDV is well known to cause potentially lethal disease among members of *Canidae*, *Mustelidae*, and *Procyonidae*. Recently, CDV has been recognized as a cause of morbidity and mortality in large felids, such as lions (*Panthera leo*) in Tanzania's Serengeti National Park in 1994 [32]; lions, tigers (*Panthera tigris*), leopards (*Panthera pardus*), and a jaguar (*Panthera onca*) in North American zoos in 1991–1992 [3]; and one Siberian tiger (*Panthera tigris altaica*) in Pokrovka, Russia, in 2004 [31]. Furthermore, CDV infection also has been reported in many non-carnivorous species, including collared peccaries (*Tayassu tajacu*) in Arizona [2] and non-human primates (*Macaca fuscata, Macaca fascicularis, Macaca mulatta*) [24, 30, 34, 39].

Lethal CDV infections in wild animals have occurred worldwide. For instance, in the Serengeti, 30% of African lions died of CDV infection [32]; and in California, the number of island foxes (*Urocyon littoralis catalane*) declined precipitously, with an approximate 95% reduction, following CDV infection [35]. In Japan, some epidemics of CDV have been reported among wild animals. In Wakayama Prefecture, 8 raccoon dogs (*Nyctereutes procyonoides*) and one weasel (*Mustela itatsi*) died of CDV infection between April 2007 and January 2008 [15]. In Kochi Prefecture, masked palm civets (*Paguma larvata*), Japanese badgers (*Meles meles*), and a large number of raccoon dogs have died of CDV infection since 2005 [37, 40].

In this study, we surveyed an apparent CDV epidemic in wild raccoon dogs and zoo-housed tigers in and around one safari-style zoo in Japan.

MATERIALS AND METHODS

Animals: Starting in December 2009, several wild raccoon dogs were found dead or dying in and around a safari-style zoo in Japan. From the end of January through February 2010, 12 tigers in the zoo exhibited diarrhea, vomiting, abdominal distress, and respiratory distress. Nine out of twelve sick tigers eventually recovered, but one of the tigers died with neuropathy on March 11, 2010. In addition, two diseased tigers also died on January 21 and February 5, 2010, although no post-mortem examination was performed.

*CORRESPONDENCE TO: MAEDA, K., Laboratory of Veterinary Microbiology, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan.
e-mail: kmaeda@yamaguchi-u.ac.jp

Table 1. Detection of CDV in fecal samples collected from tigers with diarrhea

ID	Sex	Age	Date	PCR	
				F gene	N gene
M	♂	5 years	2010.2.11	+	N.D.[a)]
			2010.2.12	+	N.D.
			2010.2.21	–	+
K	♂	5 years	2010.2.21	+	N.D.
H	♂	12 years	2010.2.22	+	+
S	♀	6 years	2010.2.13	+	+
			2010.2.14	+	N.D.
O	♀	6 years	2010.2.16	+	+
U	♀	5 years	2010.2.20	–	+
			2010.2.22	–	–
T	♂	1 year	2010.2.22	+	N.D.

a) N.D.; Not done, –; no band detected, +; rational size of band detected.

Tissue samples: Tissue samples were collected for virus isolation from four raccoon dogs and were analyzed to identify the causative agent. Fecal samples were collected from seven diseased tigers, four males and three females (Table 1). Tissue samples from the dead tiger were collected for virus isolation and histopathology.

Serum samples: Serum samples were collected from three healthy lions in the zoo after the tiger infection episode, and from nine wild bears that were captured from September 4 to October 29, 2010 in Yamaguchi Prefecture.

Cells: A72/cSLAM and CRFK/cSLAM cells expressing canine signaling lymphocyte activation molecule (SLAM) [25] were grown (at 37°C in 5% CO_2) in Dulbecco's modified Eagle's medium (DMEM: GIBCO, Grand Island, NY, U.S.A.) supplemented with 10% heat-inactivated fetal calf serum (FCS, HyClone®, Ottawa, Canada), 100 U/m*l* penicillin, and 100 μg/m*l* streptomycin (GIBCO).

Virus isolation: Swabs and fecal samples were mixed with 2 m*l* of DMEM containing antibiotics and then centrifuged at 2,000 × *g* for 15 min at 4°C. The supernatants were filtered through 0.45-μm filters (Millipore, Bedford, MA, U.S.A.). Then, A72/cSLAM cells were inoculated with filtrates and incubated until cytopathic effect (CPE) was observed.

Histopathology and immunostaining: Collected tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (3 μm thick) were stained with hematoxylin and eosin (HE). Separate paraffin sections were examined by immunohistochemistry (IHC) using a polyclonal antibody against CDV (Ikeda strain) [27]. Secondary reactions were performed with a peroxidase-conjugated Histofine-Simple stain kit (Simplestain MAX-PO; Nichirei, Tokyo, Japan), and visualized with 3,3'-diaminobenzidine and H_2O_2. IHC slides were counterstained with Mayer's hematoxylin. For negative controls, the primary antibody was omitted.

Sequence analysis of hemagglutinin (H) gene: RNA was extracted from virus-infected cells using QIAGEN RNA Mini kit (QIAGEN, Hilden, Germany) and reverse-transcription (RT) was carried out with random 9-mer primer using TaKaRa RNA LA PCR™ kit (AMV) Ver.1.1 (Takara, Shiga, Japan) at 30°C for 10 min, 42°C for 30 min, 70°C for 15 min and 5°C for 5 min. H gene was amplified using primers, CDV-HR (5'-AGA TGG ACC TCA GGG TAT AG-3') and CDV-HF (5'-AAC TTA GGG CTC AGG TAG TC-3') [8] at 94°C for 2 min and 30 cycles consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 3 min, followed by a final extension at 72°C for 15 min. The amplified products were purified by QIAquick PCR Purification kit (QIAGEN) and nucleotide sequences were directly determined by ABI sequence analyzer ABI 310 collection autosequencer (Applied Biosystem, Carlsbad, CA, U.S.A.). For sequence analysis of H gene, primers CDV-HF, CDV-HR, 204b (5'-GAA TTC GAT TTC CGC GAT CTC C-3'), 232b- (5'-TAG GCA ACA CCA ATA ATT TRG ACT C-3') [20], 1F (5'-AGG TAT GTA CTA TAG CAG TG-3'), 2F (5'-TAG TAA CCT GGA TGG TGC CT-3'), 3F (5'-CCA GGG AAT CAA GTG GAA AT-3'), 4F (5'-TCG AAC TCC AGT GAT GGC AA-3'), 1R (5'-AGG CAC CAT CCA GGT TAC TA-3'), 2R (5'-CAC TGC TAT AGT ACA TAC CT-3'), and 3R (5'TTT TGA CCC CAA CTG CAT CG-3') were used.

Sequence analysis of signal peptide region of fusion (F) protein: To amplify the signal peptide region of F gene, RT-PCR was performed using primers CDV-4713F (5'-TCG CCT CTA GGA ATC TCA CT-3') and CDV-5668R (5'-GCA GTG ATT TGT GCA GCT GT-3') at 94°C for 2 min and 30 cycles consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 1 min, followed by a final extension at 72°C for 15 min. The amplified products were purified and then analyzed by direct sequencing using primers CDV-4713F, CDV-5668R, 5275F (5'-AAC TCA GGC TCT CAG TGC A-3') and 5283R (5'-TGC ACT GAG AGC CTG AGT T-3').

Phylogenetic analysis: Homologies among our isolates and other strains of CDV, including vaccine strains deposited in Genbank, were analyzed using the GENETYX® Ver, 8. The evolutionary history was inferred using the neighbor-joining method [33]. Phylogenetic analyses were conducted in MEGA4.

Detection of CDV genes in fecal samples: RNA was extracted from fecal samples of 7 tigers using QIAamp®Viral RNA Mini Kit (QIAGEN) and RT was carried out as described above. Genes encoding signal peptides of F protein were amplified as described above and second PCR was performed using TaKaRa Ex Taq (Takara) and primers CDV-4713F and CDV-5283R as described above for the F gene. Genes encoding partial nucleocapsid (N) protein were amplified using primers, PP-I p1 (5'-ACA GGA TTG CTG AGG ACC TAT-3') and PP-I p2 (5'-CAA GAT AAC CAT GTA CGG TGC-3') at 94°C for 1 min and 40 cycles consisting of denaturation at 94°C for 1 min, annealing at 59.5°C for 2 min, and extension at 72°C for 1 min, followed by final extension at 72°C for 5 min [10]. PCR products were electrophoresed on 2% agarose gel and stained with ethidium bromide. This PCR for the N gene was reported to be very sensitive to detect from clinical samples [10]. Amplified fragments were purified by QIAquick PCR Purification kit (QIAGEN) to determine nucleotide sequences.

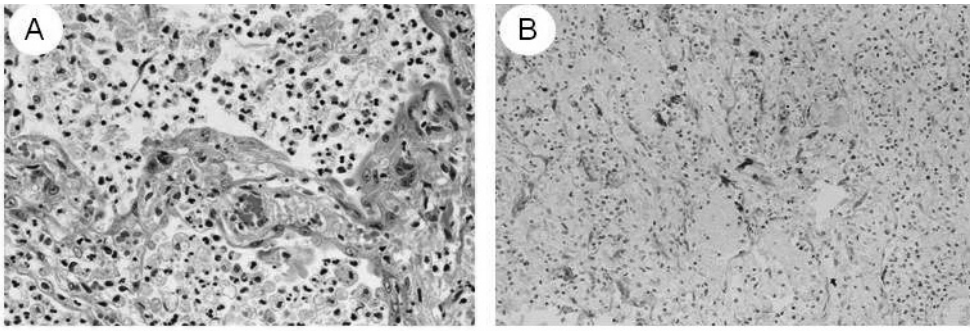

Fig.1. Section of lung lesion containing eosinophilic inclusion bodies and syncytium. (A) Hematoxylin and eosin (HE) staining. (B) IHC staining for CDV antigen.

Virus-neutralization (VN) test: VN test to KDK-1 [23] was carried out by 75% plaque-reduction neutralization test ($PRNT_{75}$) using our established cell line, CRFK/cSLAM [26]. In order to determine VN titers of the sera of lions and bears, sera were diluted to 1:5 and then serially two-fold diluted with DMEM containing 2% FCS. Diluted sera were mixed with equal volumes of virus solution containing 100 plaque-forming units (PFUs) of KDK-1, followed by incubation at 37°C for 1 hr. Then, 50 μl of mixtures were added to each well of 24-well plates (Sumilon, Tokyo, Japan) containing subconfluent CRFK/cSLAM, and the plate was incubated at 37°C for 1 hr, washed twice with DMEM without FCS, and overlaid with DMEM containing 0.8% agarose and 10% FCS. Plates then were incubated at 37°C in 5% CO_2 for 3–4 days. Cells were fixed with 5% buffered formaldehyde for 1 hr and agarose layers were removed. After staining with crystal violet, plaques were counted. VN titer was expressed as the highest dilution of serum that reduced plaques by more than 75% in comparison with control wells without serum.

RESULTS

CDV epidemic in wild raccoon dogs and bred tigers: Since December 2009, several wild raccoon dogs were found to be dead or under anesthesia in and around one safari-style zoo in Japan. Starting at the end of January 2010, 12 of 22 tigers in the zoo exhibited diarrhea, vomiting, and respiratory diseases. Two of the diseased tigers also died during this outbreak, but tissue samples were not obtained from these two tigers, nor was a cause of death determined. Another 10 of the affected tigers seemed to recover by the end of February, but one of the tigers developed a neurological disorder and died on March 11, 2010.

Virus isolation from wild raccoon dogs and the tiger: Three CDV strains, designated Yamaguchi/RD/091204, Yamaguchi/RD/091207, and Yamaguchi/RD/091209, were isolated from swabs of trachea of the raccoon dogs that died on December 4, 7 and 9, 2009, respectively. A fourth CDV strain, designated Yamaguchi/RD/091216, was isolated from the brain of the raccoon dog that died on December 16, 2009. A fifth CDV strain (designated Yamaguchi/WT/100311) was isolated from a lung of the tiger that died on March 11, 2010. These viruses were confirmed as CDV by RT-PCR and sequence analysis.

Histopathology in lung of the tiger: Histopathological changes in the dead tiger were examined (Fig. 1). A diffuse interstitial pneumonia with thick alveolar septa, marked hyperplasia of type II pneumocytes and many syncytial cells were observed. A neutrophilic exudate was superimposed on the proliferative alveolar lesion. In alveoli, there were increased number of alveolar macrophages, edema, fibrin exudation and necrotic epithelial cells and neutrophils (Fig.1A). Most bronchiolar epithelium detached from bronchiolar wall and disappeared. Numerous eosinophilic inclusion bodies mainly existed in alveolar epithelial syncytial cells and CDV antigen was detected in alveolar epithelium (Fig.1B). Inclusion bodies were also found within degenerative intestinal epithelium cells.

Detection of CDV genes from fecal samples: During CDV outbreak in tigers from February 11 to 22, fecal samples of the seven tigers were collected at various days after onset of diarrhea (Table 1). Samples were analyzed by RT-PCR for the presence of CDV RNA, specifically a 245-bp segment of the N gene and/or a 956-bp segment (via semi-nested RT-PCR) of the signal peptide region of the F gene. The result showed that CDV was detected from all examined tigers (Table 1). The nucleotide sequences of the amplified products (245 bp) were identical among fecal sample. The representative sequence of the N gene in this epidemic was deposited to DNA Data Bank of Japan (DDBJ) for Yamaguchi/WT/100311 (Accession no. AB626080). To amplify the gene (956 bp) containing the signal peptide region of F protein, RT-PCR was carried out using primers CDV-4713F and CDV-5668R for amplification. In the 1st PCR, CDV RNA was detected in 4 of 11 samples (4/7 tigers). The result of semi-nested PCR using primers CDV-4713F and -5283R (571 bp) showed that CDV was detected in 8 of 11 samples (6/7 tigers) (Table 1). Sequence analysis of the signal peptide region (405 bp) of F gene showed the sequence from one tiger ID S on February 14 was identical with those of Yamaguchi/WT/100311 (Accession no. AB619777), Yamaguchi/RD/091204 (Accession no. AB619776) and Yamaguchi/RD/091216.

Nucleotide sequences of H genes: To analyze the homo-

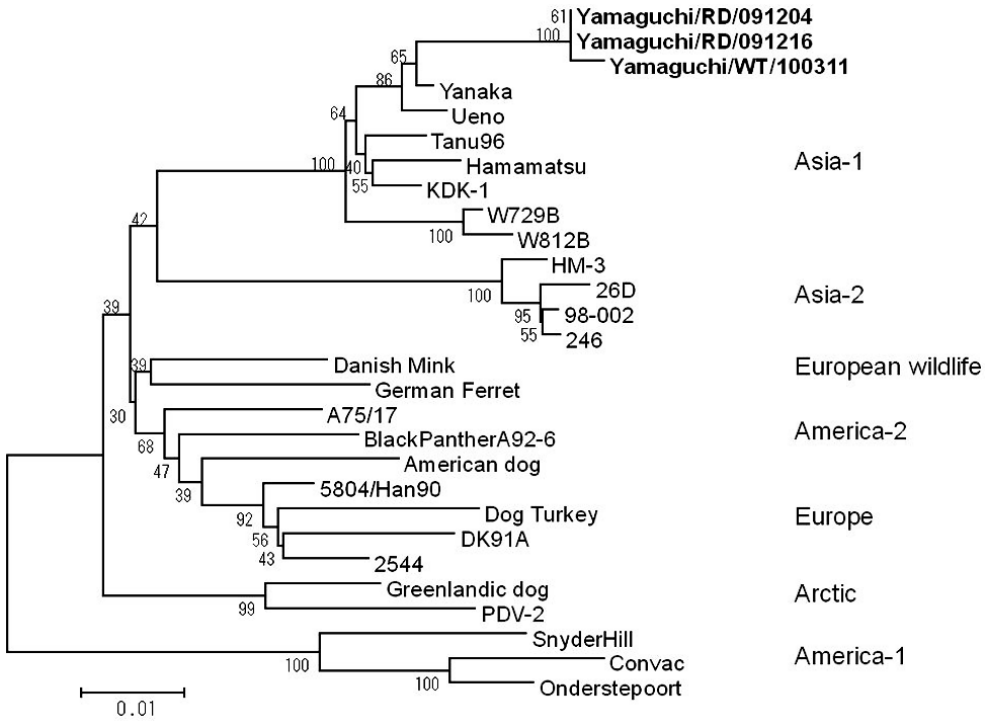

Fig. 2. Phylogenetic tree of CDV strains based on amino acid sequence of the hemagglutinin protein. Accession numbers of the sequences used are BAA19586 (Yanaka), BAA19584 (Ueno), BAA19585 (Hamamatsu), BAA33740 (Tanu96), BAA84209 (KDK-1), BAK41514 (W729B), BAK41513 (W812B), CAA87688 (Danish Mink), CAA59358 (German Ferret), AAD18008 (A75/17), CAA90879 (Black Panther A92-6), CAA87691 (American dog), CAA59359 (5804/Han90), AAM11476 (Dog Turkey), AAQ05829 (DK91 A), CAB01252 (2544), BAB39167 (HM-3), BAB39166 (26D), BAA84208 (98-002), CAA87689 (Greenlandic dog), CAA59357 (PDV-2), AAG15490 (SnyderHill), CAA84626 (Convac), AAK54669 (Onderstepoort). The scale at the bottom indicates the units of the number of amino acid substitutions per site.

genicity among strains isolated by cell culture, 2,022-bp fragments containing the H gene were amplified from Yamaguchi/RD/091204, Yamaguchi/RD/091216, and Yamaguchi/WT/100311 by RT-PCR using primers CDV-HF and CDV-HR, and the nucleotide sequences were determined by direct sequencing method and deposited to DDBJ for Yamaguchi/WT/100311 (Accession no. AB619774) and Yamaguchi/RD/091204 (Accession no. AB619775). All H genes consisted of 1824 bp encoding 607 amino acids. The predicted H protein sequences of the two isolates from raccoon dogs were identical, but the H gene of Yamaguchi/WT/100311 was predicted to encode a H protein with changes at amino acid residues 165 and 272 (99.7% identity at the protein level) (data not shown).

Comparison of H proteins among CDV strains: The deduced amino acid sequences of H proteins were compared. A substitution from tyrosine (Y) to histidine (H) at the position of 549, which was speculated to be related to CDV adaptation to wild carnivores [21], has been observed in many Japanese isolates from wild animals [15], but this Y549H substitution was not observed in CDV isolates analyzed in the present study (Yamaguchi/WT/100311, Yamaguchi/RD/091204, and Yamaguchi/RD/091216). Amino acid sequences of our isolates showed 95.9–98.4% identity to those of other strains of genotype Asia-1, 91.8–94.2% to those of other genotypes, and 89.5–89.9% identity to that of Onderstepoort (data not shown).

Phylogenetic analysis of CDV isolates: Phylogenetic analysis of the predicted H proteins was performed to identify the evolutionary relationship among these three CDV isolates and other strains. All isolates in this CDV outbreak formed a common cluster and were classified with the Asia-1 genotype (Fig. 2).

Nucleotide sequences of the signal peptide region of F proteins: Signal peptide region (amino acid residues 1–135) of F protein is thought to be the most variable region in the CDV genome [17]. Therefore, nucleotide sequences of this region were determined and deposited to DDBJ for Yamaguchi/WT/100311 (Accession no. AB619777) and Yamaguchi/RD/091204 (Accession no. AB619776). The results revealed that the pre-peptide regions of our isolates were completely identical to each other, but shared only 90.4–94.3 and 82.2–90.4% identity (nucleotide and amino acid, respectively) with the sequences of other Japanese strains of genotype Asia-1. In particular, the identity with a major vaccine strain (Onderstepoort) was as low as 80 and 62%

(nucleotide and amino acid, respectively) (data not shown).

Seroprevalence of CDV in lions bred in the zoo and in wild bears captured around the zoo: Sera from 3 lions bred in the zoo were examined by VN test using KDK-1 as described previously [23]. By this VN test, we succeeded in the detection of antibody to CDV in dogs, raccoons and many wild animal species [15, 23, 26]. Only one of the 3 lions possessed VN antibody to CDV. The VN titer was high at 1:320 (Table 2). Sera from 9 wild bears (*Ursus thibetanus*) captured in this area after this CDV outbreak also were examined by VN test; only one of the 9 samples possessed VN antibody to CDV. The VN titer from this animal was 1:40 (Table 3).

Table 2. Seroprevalence of CDV in lions in the zoo

ID	Sex	Age	Virus-neutralizing titer
S	♀	1 year	<1:10
M	♀	1 year	<1:10
H	♂	12 years	1:320

Table 3. Seroprevalence of CDV in wild bears captured in Yamaguchi Prefecture

ID	Virus-neutralizing titer
100904	<1:10
100912	1:40
100914-1	<1:10
100914-2	<1:10
101022	<1:10
101024	<1:10
101028-1	<1:10
101028-2	<1:10
101029	<1:10

DISCUSSION

Twelve tigers in the zoo exhibited diarrhea, vomiting, abdominal distress, and respiratory distress starting at the end of January, and one of the tigers died with neuropathy on March 11, 2010. By IHC analysis and virus isolation, CDV infection was etiologically involved in the death of this tiger. Furthermore, CDV RNA was detected in at least one sample from each animal between February 11 and 22, indicating that a CDV outbreak occurred among tigers in this zoo. On January 21 and February 5, another 2 of 12 diseased tigers at this site died after exhibiting diarrhea and vomiting. Although no post-mortem examination was performed, these tigers also were suspected to have been killed by CDV infection. In this zoo, a total of 22 tigers were housed in two different areas. Notably, 17 tigers that were housed together (in one of these two areas) accounted for all 12 tigers exhibiting symptoms and all 3 deaths, indicating that morbidity and mortality ratios of CDV in this group of tigers were 71 and 25%, respectively.

Raccoon dogs are highly susceptible to CDV infection, with high morbidity and mortality [18]. CDV epidemics in raccoon dogs, evidenced by animals exhibiting pneumonia and gastroenteritis, have been reported previously in Japan [18, 37, 40]. In our own previous study, we also observed 8 raccoon dogs dead by CDV infection in Tanabe City, Wakayama Prefecture [15]. In this study, several wild raccoon dogs, dead of apparent CDV infection, were discovered around the zoo within a month before a CDV outbreak among tigers housed at the zoo. Although the discovery and diagnosis of dead and dying wild animals is a challenge, raccoon dogs might be good indicators for CDV outbreaks among wild animals because of this species' high sensitivity to CDV and high population density in Japan.

To analyze antigenic variation, phylogenetic analysis of the H protein-encoding genes [5, 11, 22, 28] was performed on several of the CDV isolates obtained in this study. The predicted H proteins revealed a very close relationship (99.7% protein identity) among CDV from the raccoon dogs and the dead tiger. Furthermore, our isolates from the raccoon dogs and tiger were identical in the amino acid sequence even in the signal peptide region of F protein, which is thought to be most variable among isolates [17]. These results indicate that wild raccoon dogs and the tiger were infected with the same CDV. Therefore, based on geographical and sequential conditions, we conclude that this CDV epidemic occurred by transmission of CDV from raccoon dogs (or other wild animals around the zoo) to tigers. Although the exact routes of transmission remain unknown, two possible routes are suggested. One is that wild animals infected with CDV entered into the zoo grounds and had direct contact with the tigers, and another is that agents such as humans, food, excreta, or so on played a role as a carrier. In this safari-style zoo, the staffs carried out disinfection more carefully after detection of CDV in raccoon dogs. However, the outbreak occurred among tigers in this zoo. Therefore, we speculated that CDV-infected wild animals must have become disorder of central nervous system and invaded the zoo, even area of tigers. From the ground and water contaminated by their urine and feces containing CDV, tigers must have been infected with CDV.

Our isolates did not have a substitution from tyrosine (Y) to histidine (H) at hemagglutinin amino acid position 549, although this substitution was speculated to be related to CDV adaptation to wild carnivores [21]. In addition, recent isolates from Japanese wild animals possessed this substitution Y549H [15]. Therefore, we propose that our isolates might have originated from domestic dogs and might not have been adapted well to wild carnivores. CDV transmission among different species has been reported previously. In the case of Serengeti lions, domestic dog was considered a likely source of CDV infection [7]; in California, wild raccoons were hypothesized to transmit CDV to large cats [3]. In Japan, it was speculated that domestic dogs were the sources of CDV infection of feral masked palm civets [13].

Although genotype Asia-1 was isolated previously from dogs and wild animals in Japan [13, 14, 22], our results demonstrate that this CDV genotype also can infect felids. CDV seropositivity in one lion supports the CDV outbreak in the zoo. In Japan, anti-CDV antibody was detected in 13

of 20 lions at another zoo, although those animals exhibited no obvious clinical sign of infection [9]. In the Serengeti, frequent interactions with jackals and spotted hyenas at kills have been supposed to provide a potential mechanism for transmission to lions [7]. Moreover, seropositivity of one wild bear (*Ursus thibetanus*) captured around the zoo supports the possibility of a CDV outbreak among wild mammals around the zoo. As a predator of arctic seals, polar bear (*Ursus maritimus*) has been suggested to be susceptible to CDV infection [6]. In the current study, it is assumed that the wild bear might have been exposed by feeding on CDV-infected wild animals. These seropositivities indicated that many mammals in and around the zoo were exposed to CDV during this episode.

In recent years, CDV seems to have broadened its host range to include wild animals [12]. All over the world, CDV has led to mass mortalities in species ranging from wild canids (island fox, *Urocyon littoralis*) [35], felids (lion, *Panthera leo*) [32], phocids (Bikal seal, *Phoca siberica*) [19], viverrids (masked palm civet, *Paguma larvata*) [13], and procyonids (raccoon dogs, *Nyctereutes procyonoides*) [37, 40], to primates (crab-eating macaque, *Macaca fascicularis* and rhesus monkeys, *Macaca mulatta*) [24, 30, 34]. The increased host range of CDV suggests that this virus might represent a possible zoonosis, as indicated by its detection in non-human primates.

CDV epidemics also have threatened the continuation of the small population such as island fox [35], giant panda (*Ailuropoda melanoleuca*) [29], black-footed ferret (*Mustela nigripes*) [38] and African wild dogs (*Lycaon pictus*) [36]. Thus the expanding host range of CDV represents a challenge in the conservation of endangered animals. In the Serengeti, since CDV vaccination to domestic dogs has been adopted, CDV outbreak has not been observed in lions or hyenas [7]. Vaccines should be developed for wild and zoo animals, especially endangered species; the CDV vaccine used in dogs cannot be directly applied in other species, because other animals exhibit different sensitivities to CDV [4].

In conclusions, these results indicate a risk of CDV transmission from wild animals to zoo animals including large felids and endangered species.

ACKNOWLEDGMENTS. We thank Dr. Masami Mochizuki (Kyoritsu Seiyaku Corporation) for kindly providing the KDK-1 strain. This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Appel, M. J. 1969. Pathogenesis of canine distemper. *Am. J. Vet. Res.* **30**: 1167–1182. [Medline]
2. Appel, M. J., Reggiardo, C., Summers, B. A., Pearce-Kelling, S., Maré, C. J., Noon, T. H., Reed, R. E., Shively, J. N. and Örvell, C. 1991. Canine distemper virus infection and encephalitis in javelinas (collared peccaries). *Arch. Virol.* **119**: 147–152. [Medline] [CrossRef]
3. Appel, M. J., Yates, R. A., Foley, G. L., Bernstein, J. J., Santinelli, S., Spelman, L. H., Miller, L. D., Arp, L. H., Anderson, M. and Barr, M. 1994. Canine distemper epizootic in lions, tigers, and leopards in North America. *J. Vet. Diagn. Invest.* **6**: 277–288. [Medline] [CrossRef]
4. Appel, M. J. and Summers, B. A. 1995. Pathogenicity of morbilliviruses for terrestrial carnivores. *Vet. Microbiol.* **44**: 187–191. [Medline] [CrossRef]
5. Blixenkrone-Möller, M., Svansson, V., Appel, M., Krogsrud, P., Have, P. and Orvell, C. 1992. Antigenic relationship between field isolates of morbilliviruses from different carnivores. *Arch. Virol.* **123**: 279–294. [Medline] [CrossRef]
6. Cattet, M. R., Duignan, P. J., House, C. A. and Aubin, D. J. 2004. Antibodies to canine distemper and phocine distemper viruses in polar bears from the Canadian arctic. *J. Wildl. Dis.* **40**: 338–342. [Medline]
7. Cleaveland, S., Appel, M. G., Chalmers, W. S., Chillingworth, C., Kaare, M. and Dye, C. 2000. Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. *Vet. Microbiol.* **72**: 217–227. [Medline] [CrossRef]
8. Demeter, Z., Lakatos, B., Palade, E. A., Kozma, T., Forga'ch, P. and Rusvai, M. 2007. Genetic diversity of Hungarian canine distemper virus strains. *Vet. Microbiol.* **122**: 258–269. [Medline] [CrossRef]
9. Endo, Y., Uema, M., Miura, R., Tsukiyama-Kohara, K., Tsujimoto, H., Yoneda, K. and Kai, C. 2004. Prevalence of canine distemper virus, feline immunodeficiency virus and feline leukemia virus in captive African lions (Panthera leo) in Japan. *J. Vet. Med. Sci.* **66**: 1587–1589. [Medline] [CrossRef]
10. Frisk, A. L., Konig, M., Moritz, A. and Baumgartner, W. 1999. Detection of Canine Distemper virus nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. *J. Clin. Microbiol.* **37**: 3634–3643. [Medline]
11. Haas, L., Martens, W., Greiser-Wilke, L., Mamaev, L., Butina, T., Maack, D. and Barrett, T. 1997. Analysis of the hemagglutinin gene of current wild-type canine distemper virus isolates from Germany. *Virus Res.* **48**: 165–171. [Medline] [CrossRef]
12. Harder, T. C. and Osterhaus, A. D. 1997. Canine distemper virus-a morbillivirus in search of new hosts? *Trends Microbiol.* **5**: 120–124. [Medline] [CrossRef]
13. Hirama, K., Goto, Y., Uema, M., Endo, Y., Miura, R. and Kai, C. 2004. Phylogenetic analysis of the hemagglutinin (H) gene of canine distemper viruses isolated from wild masked palm civets (*Paguma larvata*). *J. Vet. Med. Sci.* **66**: 1575–1578. [Medline] [CrossRef]
14. Iwatsuki, K., Miyashita, N., Yoshida, E., Gemma, T., Shin, Y. S., Mori, T., Hirayama, N., Kai, C. and Mikami, T. 1997. Molecular and phylogenetic analyses of the haemagglutinin (H) proteins of field isolates of canine distemper virus from naturally infected dogs. *J. Gen. Virol.* **78**: 373–380. [Medline]
15. Kameo, Y., Nagao, Y., Nishio, Y., Shimoda, H., Nakano, H., Suzuki, K., Une, Y., Sato, H., Shimojima, M. and Maeda, K. 2012. Epizootic canine distemper virus infection among wild mammals. *Vet. Microbiol.* **154**: 222–229. [Medline] [CrossRef]
16. Lamb, R. A. and Kolakofsky, D. 2001. Paramyxoviridae: the viruses and their replication. pp. 1305–1443. *In:* Fields of Virology, 4th ed. (Knipe, D. M. and Howley, P. M. eds), Lippincott Williams & Wilkins, Philadelphia.
17. Lee, M. S., Tsai, K. J., Chen, L. H., Chen, C. H., Liu, Y. P., Chang, C. C., Lee, S. H. and Hsu, W. L. 2010. The identifi-

cation of frequent variations in the fusion protein of canine distemper virus. *Vet. J.* **183**: 184–190. [Medline] [CrossRef]
18. Machida, N., Kiryu, K., Oh-ishi, K., Kanda, E., Izumisawa, N. and Nakamura, T. 1993. Pathology and epidemiology of canine distemper in raccoon dogs (*Nyctereutes procyonoides*). *J. Comp. Pathol.* **108**: 383–392. [Medline] [CrossRef]
19. Mamaev, L. V., Visser, L. K., Belikov, S. I., Denikina, N., Harder, T., Goatley, L., Rima, B., Edginton, B., Osterhaus, A. D. and Barrett, T. 1996. Canine distemper virus in Lake Baikal seals (*Phoca sibirica*). *Vet. Rec.* **138**: 437–439. [Medline] [CrossRef]
20. Martella, V., Elia, G., Lucente, M. S., Decaro, N., Lorusso, E., Banyai, K., Blixenkrone-Moller, M., Lan, N. T., Yamaguchi, R., Cirone, F., Carmichael, L. E. and Buonavoglia, C. 2007. Genotyping canine distemper virus (CDV) by a hemi-nested multiplex PCR provides a rapid approach for investigation of CDV outbreaks. *Vet. Microbiol.* **122**: 32–42. [Medline] [CrossRef]
21. McCarthy, A. J., Shaw, M. A. and Goodman, S. J. 2007. Pathogen evolution and disease emergence in carnivores. *Proc. Biol. Sci.* **274**: 3165–3174. [Medline] [CrossRef]
22. Mochizuki, M., Hashimoto, M., Hagiwara, S., Yoshida, Y. and Ishiguro, S. 1999. Genotypes of canine distemper virus determined by analysis of the hemagglutinin genes of recent isolates from dogs in Japan. *J. Clin. Microbiol.* **37**: 2936–2942. [Medline]
23. Mochizuki, M., Motoyoshi, M., Maeda, K. and Kai, K. 2002. Complement-mediated neutralization of canine distemper virus in vitro: cross-reaction between vaccine Onderstepoort and field KDK-1 strains with different hemagglutinin gene characteristics. *Clin. Diagn. Lab. Immunol.* **9**: 921–924. [Medline]
24. Morikawa, S. 2008. Outbreak of deadly canine distemper virus infection among imported cynomolgus (crab-eating) monkeys. *Infect. Agents Surveill. Rep.* **29**: 315.
25. Nakano, H., Kameo, Y., Andoh, K., Ohno, Y., Mochizuki, M. and Maeda, K. 2009. Establishment of canine and feline cells expressing canine signaling lymphocyte activation molecule for canine distemper virus study. *Vet. Microbiol.* **133**: 179–183. [Medline] [CrossRef]
26. Nakano, H., Kameo, Y., Sato, H., Mochizuki, M., Yokoyama, M., Uni, S., Shibasaki, T. and Maeda, K. 2009. Detection of antibody to canine distemper virus in wild raccoons (*Procyon lotor*) in Japan. *J. Vet. Med. Sci.* **71**: 1661–1663. [Medline] [CrossRef]
27. Nunoya, T., Tajima, M., Ishikawa, Y., Samejima, T., Ishikawa, H. and Hasegawa, K. 1990. Occurrence of a canine distemper-like disease in aquarium seals. *Nippon Juigaku Zasshi* **52**: 469–477. [Medline] [CrossRef]
28. Orvell, C., Bilxenkrone-Moller, M., Svansson, V. and Have, P. 1990. Immunological relationships between phocid and canine distemper virus studied with monoclonal antibodies. *J. Gen. Virol.* **71**: 2085–2092. [Medline] [CrossRef]
29. Qin, Q., Li, D., Zhang, H., Hou, R., Zhang, Z., Zhang, C., Zhang, J. and Wei, F. 2010. Serosurvey of selected viruses in captive giant pandas (*Ailuropoda melanoleuca*) in China. *Vet. Microbiol.* **142**: 199–204. [Medline] [CrossRef]
30. Qiu, W., Zheng, Y., Zhang, S., Fan, Q., Liu, H., Zhang, F., Wang, W., Liao, G. and Hu, R. 2011. Canine distemper outbreak in rhesus monkeys, China. *Emerg. Infect. Dis.* **17**: 1541–1543. [Medline]
31. Quigley, K. S., Evermann, J. F., Leathers, C. W., Armstrong, D. L., Goodrich, J., Duncan, N. M. and Miquelle, D. G. 2010. Morbillivirus infection on a wild Siberian tiger in the Russian Far East. *J. Wildl. Dis.* **46**: 1252–1256. [Medline]
32. Roelke-Parker, M. E., Munson, L., Packer, C., Kock, R., Cleaveland, S., Carpenter, M., O'Brien, S. J., Pospischil, A., Hofmann-Lehmann, R. and Lutz, H. 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* **379**: 441–445. [Medline] [CrossRef]
33. Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425. [Medline]
34. Sun, Z., Li, A., Ye, H., Shi, Y., Hu, Z. and Zeng, L. 2010. Natural infection with canine distemper virus in hand-feeding Rhesus monkeys in China. *Vet. Microbiol.* **141**: 374–378. [Medline] [CrossRef]
35. Timm, S. F., Munson, L., Summers, B. A., Terio, K. A., Dubovi, E. J., Rupprecht, C. E., Kapil, S. and Garcelon, D. K. 2009. A suspected canine distemper epidemic as the cause of a catastrophic decline in Santa Catalina Island foxes (Urocyon littoralis catalinae). *J. Wildl. Dis.* **45**: 333–343. [Medline]
36. van de Bildt, M. W., Kuiken, T., Visee, A. M., Lema, S., Fitzjohn, T. R. and Osterhaus, A. D. 2002. Distemper outbreak and its effect on African wild dog conservation. *Emerg. Infect. Dis.* **8**: 211–213. [Medline] [CrossRef]
37. Watabe, T. and Yoshizawa, M. 2006. The outbreak of death frequent occurrence of the wild raccoon dog by canine distemper. *J. Environ. Dis.* **15**: 11–14 (in Japanese).
38. Williams, E. S. and Thorne, E. T. 1996. Infectious and parasitic diseases of captive carnivores, with special emphasis on the black-footed ferret (*Mustela nigripes*). *Rev. Sci. Tech.* **15**: 91–114. [Medline] [CrossRef]
39. Yoshikawa, Y., Ochikubo, F., Matsubara, Y., Tsuruoka, H., Ishii, M., Shirota, K., Nomura, Y., Sugiyama, M. and Yamanouchi, K. 1989. Natural infection with canine distemper virus in a Japanese monkey (*Macaca fuscata*). *Vet. Microbiol.* **20**: 193–205. [Medline] [CrossRef]
40. Yoshizawa, M. and Watabe, T. 2007. The canine distemper outbreak situation of mammals in Kochi City and the outskirts. *Kagawa Seibutsu* **34**: 63–67 (in Japanese).