NOTE *Pathology*

Canine Distemper Virus Infection in Fennec Fox (*Vulpes zerda*)

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ABSTRACT. Fifteen 8-month-old fennec foxes imported from Sudan showed fever, mucopurulent ocular discharge, diarrhea, severe emaciation, seizures, and generalized ataxia, and died. Three of the 15 animals were presented for diagnostic investigation. Severe dehydration, brain congestion, and gastric ulcers were observed in all animals. In one animal, the lungs had failed to collapse and were multifocally dark red in appearance. Histopathologically, there were lymphohistiocytic meningoencephalitis with malacia, mild interstitial pneumonia, lymphoid depletion of lymphoid tissues and organs, and intestinal villous atrophy with intralesional coccidia. There were many intracytoplasmic and/or intranuclear inclusion bodies in the epithelial cells of the medullary velum, lungs, liver, kidneys, trachea, pancreas, stomach, gall bladder, urinary bladder, and ureters, and in macrophages of malacia foci and lymphocytes and macrophages of lymphoid organs. Additionally, intestinal coccidia were confirmed to be *Isospora* species by a fecal test. To our knowledge, this is the first report of canine distemper with intestinal coccidiosis in fennec fox.

KEY WORDS: canine distemper, coccidium, fennec fox, meningoencephalitis, *Vulpes zerda*.

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Canine distemper (CD), caused by the morbillivirus of the paramyxovirus family, frequently causes serious problems in dogs, such as the respiratory, gastrointestinal, integumentary and nervous symptoms. Since CD was firstly reported by Carré, canine distemper virus (CDV) infection has been demonstrated in a wide range of carnivores, such as Ailuridae (red panda) [7], Canidae (dogs, wolves and foxes) [1, 5, 6, 10, 13, 14], Mephitidae (skunks) [27], Mustelidae (ferrets, minks, otters, and badgers) [5, 19, 23, 24], Procyonidae (raccoons) [14], Ursidae (giant pandas) [20], Felidae (lions, tigers, and leopards) [4, 21], Hyaenidae (hyenas) [2] and Viverridae (binturongs and civet) [15, 18]. CD-like epidemics have also been reported in seals, dolphins and porpoises [26].

The fennec fox (*Vulpes zerda*) is classified into the family Canidae of the order Carnivora. Although CDV susceptibility has been suspected in the fennec foxes [9], no clinical case of natural CDV infection has been documented. In this study, we describe a natural CDV infection in fennec foxes imported from Sudan.

Fifteen 6- to 8-month-old fennec foxes were imported from the same merchant and moved to the zoological garden for quarantine and acclimation. Two foxes (cases Nos. 1 and 2) that showed fever, mucopurulent ocular discharge, severe emaciation, seizures, and generalized ataxia, died the next day after arriving at the airport. The others developed fever, diarrhea, anorexia and ocular exudates, and were treated with antibiotic and anti-inflammatory therapy. However, their clinical signs gradually failed and they began to exhibit neurologic symptoms such as ataxia and seizures. Most of the foxes died spontaneously within a few days after the neurologic signs appeared (Table 1). One dead (case No. 13) and two moribund foxes (cases Nos. 14 and 15) of the 15 animals were submitted for histopathology and immunohistochemistry; the moribund foxes were humanely euthanized for necropsy. Organs and tissues such as brain, lungs, heart, liver, spleen, kidneys, pancreas, trachea, stomach, intestine, gall bladder, urinary bladder, and lymph nodes were fixed in 10% buffered formalin and embedded in paraffin wax. Sections (4 μm) were cut and stained with hematoxylin and eosin (HE) for routine histologic examination. Additional sections were immunostained with an anti-CDV antibody (AbD Serotec, Oxford, U.K.) using a fully automatic immunohistochemical system (Ventana Discovery XT; Ventana Medical Systems Inc., Tucson, AZ, U.S.A.) with the DAB Detection System (Ventana) according to the manufacturer's instructions. Briefly, paraffin sections were deparaffinized at 75°C for 8 min, treated with protease 1 for 8 min at 37°C, and exposed to the mouse anti-CDV antibody (1:500 dilution) in antibody diluent (Dako North America, Inc., Carpinteria, CA, U.S.A.) for 32 min at 37°C. The universal secondary antibody, detection reagent, and DAB chromogen (Ventana) were then applied sequentially at 37°C. The sections were counterstained with hematoxylin and bluing reagent (Ventana) and cover-slipped for microscopic examination. Fresh tissue samples and intestinal contents were also collected for bacteriologic and parasitic examination, respectively. In addition, portions of the tissue samples such as brain, lungs, and spleen of 3 submitted animals (Nos. 13–15) were used for the amplification of viral sequence by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted using QIAamp viral RNA Mini kit (QIAGEN Inc.,

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Table 1. Clinical signs and results of RT-PCR in fennec fox

No. of animals	Sex	Onset of symptom	Clinical signs	Date of death	RT-PCR	Remarks
1	F	Unknown[a)]	ocular discharge, diarrhea, emaciation, ataxia, seizures, death	Day 2[b)]	NT[c)]	
2	F	Unknown	ocular discharge, diarrhea, emaciation, ataxia, seizures, death	Day 2	NT	
3	M	Unknown	ocular discharge, diarrhea, emaciation, seizures, death	Day 3	NT	
4	F	Unknown	ocular discharge, anorexia, diarrhea, emaciation, ataxia, seizures, death	Day 6	NT	
5	M	Unknown	ocular discharge, anorexia, diarrhea, emaciation, ataxia, death	Day 7	NT	
6	M	Unknown	ocular discharge, anorexia, diarrhea, emaciation, seizures, death	Day 7	NT	
7	M	Day 2[b)]	ocular discharge, anorexia, diarrhea, emaciation, seizures, death	Day 10	NT	
8	F	Day 7	ocular discharge, anorexia, diarrhea, emaciation, ataxia, death	Day 29	Positive[d)]	
9	F	Day 8	ocular discharge, diarrhea, emaciation, seizures, death	Day 33	Positive	
10	M	Day 23	ocular discharge, anorexia, diarrhea, emaciation, seizures, death	Day 40	Positive	
11	M	Day 30	ocular discharge, diarrhea, emaciation, seizures, death	Day 42	Positive	
12	F	Day 23	ocular discharge, diarrhea, emaciation, seizures, death	Day 42	Positive	
13	M	Day 29	ocular discharge, anorexia, diarrhea, emaciation, ataxia, seizures, death	Day 43	Positive	Necropsy
14	F	Day 28	ocular discharge, diarrhea, emaciation, ataxia, seizures, moribund	–	Positive	Necropsy
15	F	Day 31	ocular discharge, diarrhea, emaciation, seizures, moribund	–	Positive	Necropsy

a) Severe clinical signs were shown from arrival day.
b) Arrival day was represented as Day 1.
c) NT, not tested.
d) Samples were collected at Day 28.

Germantown, MD, U.S.A.). The primer sets were designed in the H gene regions of a CDV genome sequence and the sequences were 5'-GGTCCGGTTATACTGAACGG-3' (sense) and 5'-TCAAGGTTTTGAACGGTTAC-3' (antisense). RT-PCR was performed using a one step RT-PCR kit (QIAGEN Inc.) and composed of 42°C for 30 min, 94°C for 15 min and then 35 cycles of 94°C for 30 sec, 51°C for 30 sec, and 72°C for 40 sec. Amplification products were electrophoresed on a 2.0% agarose gel, and observed under UV illumination.

At necropsy, 3 submitted animals (Nos. 13–15) were severely emaciated and dehydrated (Fig. 1), and their brains were congested. In one of the three animals, the lungs had failed to collapse and were multifocally dark red in appearance. Ulcerative foci were scattered in the stomach. Histopathologically, lymphohistiocytic perivascular cuffings and malacia were observed in cerebrum (Fig. 2), and malacia, vacuolation and lymphohistiocytic meningoencephalitis were present in the cerebellum (Fig. 3). There was mild bronchointerstitial pneumonia in the lungs. Lymphoid depletion and lymphopolysis were seen in the spleen, lymph nodes and Peyer's patch of intestines, suggesting the immunodepressive status of the affected animals. Intestinal villi were atrophic, and lymphocytes, macrophages, and eosinophils were infiltrated in the lamina propria of small intestine. Numerous meronts, macrogametocytes and developing oocysts were present in the lamina propria, epithelia of villi and crypts of the jejunum (Fig. 4), ileum, cecum and colon. In particular, oocysts were most frequently observed in ileum. Additionally, sporulated oocysts containing two sporocysts, suggesting *Isospora* sp., were identified by a fecal test (Fig. 4, insert). No histopathologic findings were noted in other organs and no significant bacteria were isolated from tissue samples such as lungs and intestine. Eosinophilic intracytoplasmic and/or intranuclear inclusion bodies were observed in the epithelial cells of the medullary velum, lungs, liver, kidneys, trachea, pancreas, stomach, gall bladder, urinary bladder, and ureter, and in macrophages of malacia foci and lymphocytes and macrophages of spleen, lymph nodes and Peyer's patch. CDV antigens were immunohistochemically detected in cells with or without inclusion bodies (Fig. 5). Also, positive signals were detected by PCR in 3 submitted animals.

The Canidae family is classified into two tribes; the Canini tribe, which includes dogs, wolves, jackals, and coyotes, and the Vulpini tribe, which includes foxes. CD has been reported in most of animals belong to Canini tribe and Vulpini tribe [11] whereas CDV infection has not previously been demonstrated in fennec fox of Vulpini tribe. In this study, we demonstrated the first natural outbreak of disease caused by CDV in fennec fox. In dogs, clinical signs of a CDV systemic infection are commonly related to the respiratory and gastrointestinal systems, and include fever, conjunctivitis, pneumonia, depression, anorexia, vomiting and diarrhea [8]. Neurologic disease may developed 1–4 weeks after recovery from systemic infection. Although systemic infection was detected, neurological symptoms, such as ataxia and seizures, were prominent in our cases, which findings were different from typical clinical signs in domestic dogs. Similar neurologic disease has also been demonstrated in javelinas [3] and lions [21]. Immunoreactivity to CDV in various cells of the brain in our cases suggested the spread of CDV in the cerebrospinal fluid. CDV infect in the CNS mainly occurs via the hematogenous route [16] and spread in the cerebrospinal fluid [25]. Viral neuroinvasion via the olfactory nerves also occurs [22].

Cellular and humoral immunity are suppressed or inhibited by damage to the lymph organs, with lymphocyte loss and leukocytopenia in dogs with CDV [17], and affected animals become highly susceptible to other opportunistic infectious diseases, such as toxoplasmosis [10], cryptosporidiosis [10], yersiniosis [6] and listeriosis [6]. Canine intes-

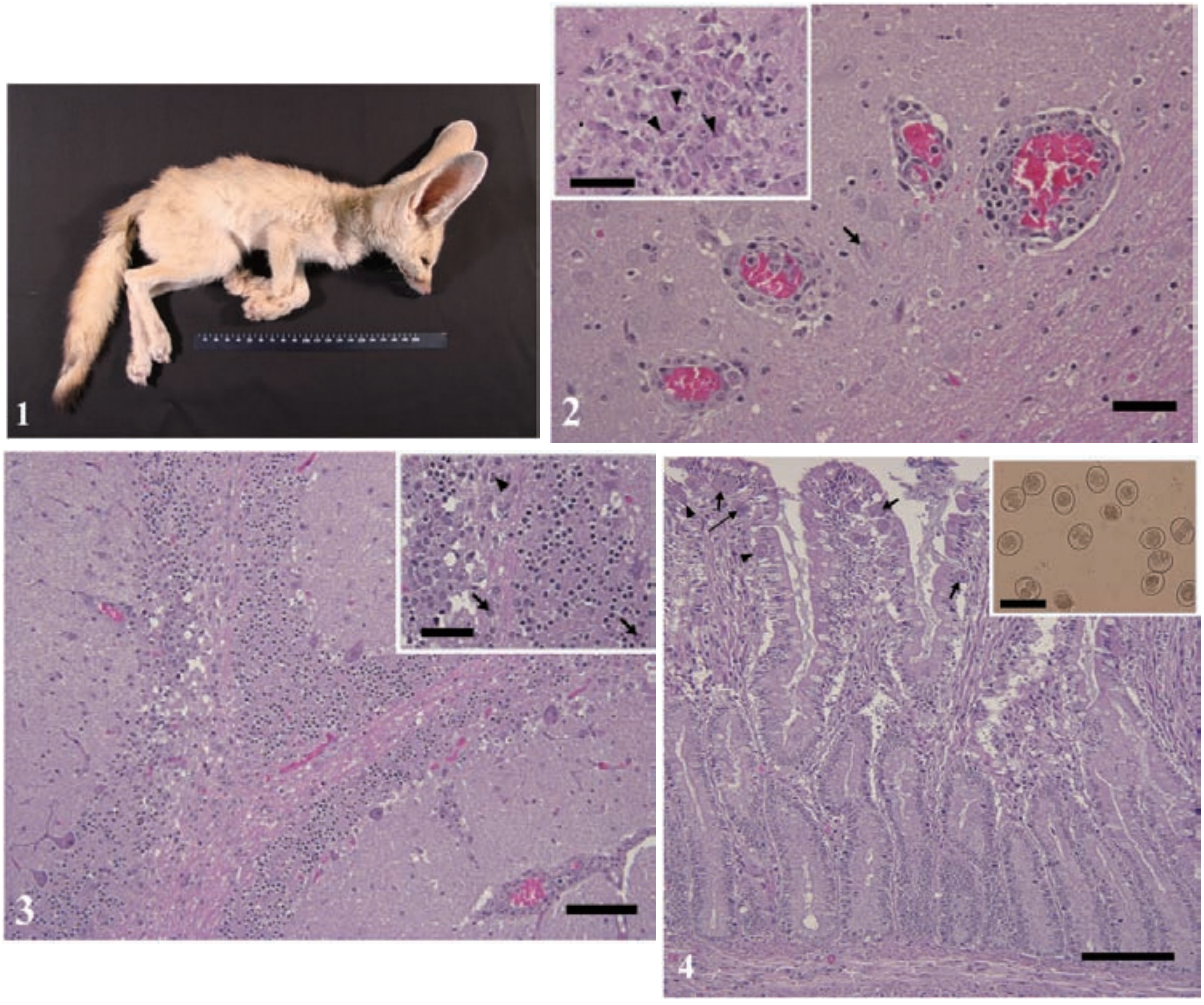

Fig. 1. External appearance. Note ocular discharge, severe emaciation, and dehydration.
Fig. 2. Cerebrum. Lymphocytes and macrophages have infiltrated around blood vessels and a linear eosinophilic intranuclear inclusion body can be seen in the neuron (arrow). Hematoxylin and eosin. Bar=100 μm. Insert: Eosinophilic intracytoplasmic inclusion bodies are present in malacic area (arrowheads). Bar=100 μm.
Fig. 3. Cerebellum. Malacia and vacuolation can be seen in granular cell layer and a few lymphocytes are infiltrated around blood vessels and in the meninges. Hematoxylin and eosin. Bar=200 μm. Insert: Eosinophilic intracytoplasmic inclusion body (arrowhead) and intranuclear inclusion bodies (arrows) are seen in granular cell layer. Bar=100 μm.
Fig. 4. Jejunum. Several stages of coccidian, including meronts (arrowheads), gamonts (short arrows), and a macrogametocyte (long arrow) are seen in the lamina propria. Hematoxylin and eosin. Bar=200 μm. Insert: The oocysts are either unsporulated or are in the two-sporoblast stage. Bar=100 μm.

tinal coccidiosis causes diarrhea, and is a common cause of infections in puppies, young dogs and immunosuppressed dogs, although healthy animals typically show no clinical signs [12]. Although the pathogenicity of intestinal coccidiosis has not been demonstrated in foxes, we suggest that coccidia could be pathogenic in fennec fox immunosuppressed by CDV, or stressed by environmental conditions, such as capture and long-distance transportation.

In the future, in case of the introduction of susceptible wild animals by CDV, vaccination programmes should be considered prior to animal import.

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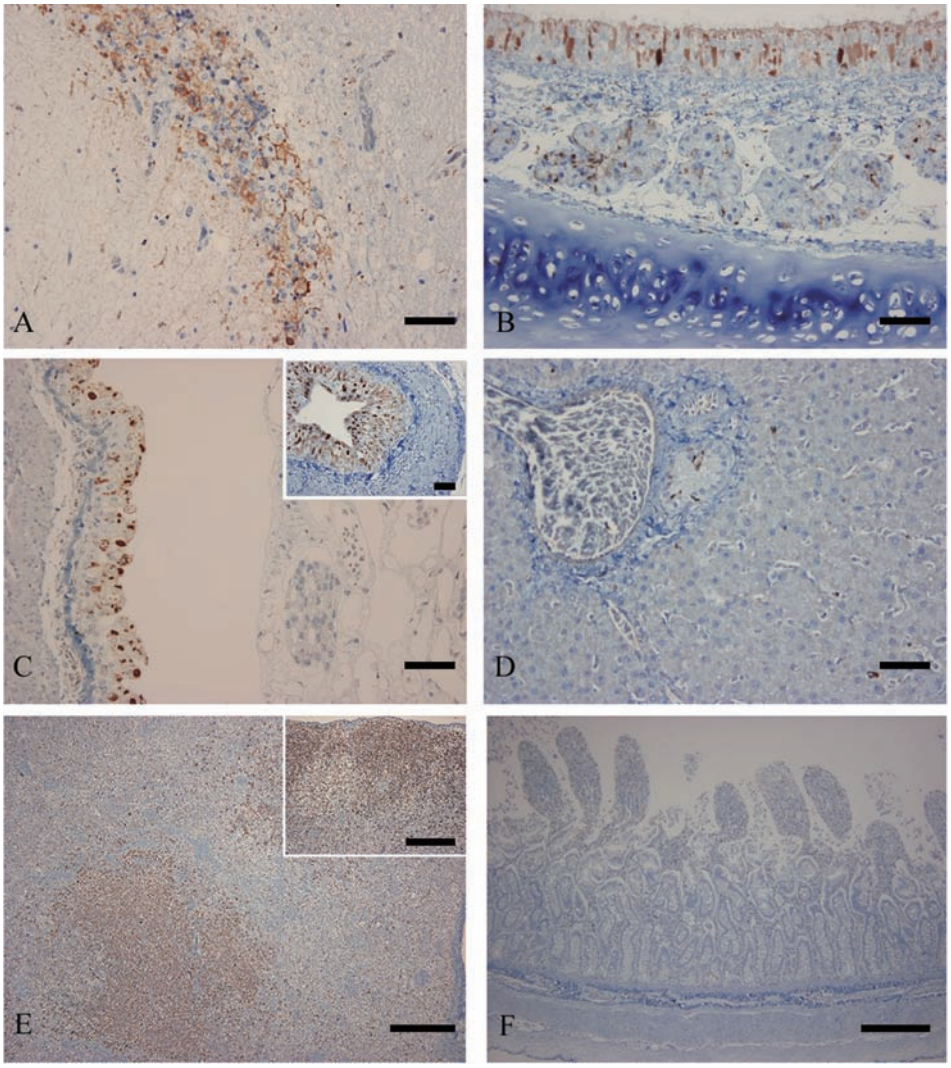

Fig. 5. Immunohistochemical staining for canine distemper and DAB chromogen. A. Cerebellum. Immunoreactivity is seen mostly in the granular cell layer around the malacia and in the molecular layer. Immunoperoxidase with hematoxylin and bluing reagent counterstain. Bar=100 μm. B. Lung. Immunopositive reactions are seen in epithelial layer and submucosal gland of the bronchus. Immunoperoxidase with hematoxylin and bluing reagent counterstain. Bar=100 μm C. Kidney. Viral antigens are detected in the transitional epithelia of the renal pelvis. Immunoperoxidase with hematoxylin and bluing reagent counterstain. Bar=100 μm. Insert: Ureter. Viral antigens are seen in the transitional epithelia. Bar=100 μm. D. Liver. Immunopositive reactions are seen in epithelia of the bile duct and kupffer cells. Immunoperoxidase with hematoxylin and bluing reagent counterstain. Bar=100 μm. E. Spleen. Immunopositive reactions are observed in lots of lymphoid cells. Immunoperoxidase with hematoxylin and bluing reagent counterstain. Bar=200 μm. Insert: Lymph node. Viral antigens are detected in many lymphoid cells. Bar=200 μm. F. Small intestine. Immunopositive reactions are observed in mononuclear cells of lamina propria and a few enterocytes. Immunoperoxidase with hematoxylin and bluing reagent counterstain. Bar=200 μm.

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