SHORT COMMUNICATIONS

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Morbillivirus Infection in a Wild Siberian Tiger in the Russian Far East

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ABSTRACT: We report the first documented case of morbillivirus infection in a wild, free-ranging Siberian tiger (*Panthera tigris altaica*). The tigress entered a small village in the Russian Far East in an ambulatory but stuporous state with no apparent recognition or fear of humans. Her condition progressed rapidly with neurological signs, anorexia, and ultimately death. Histologic lesions included vacuolated to malacic white matter in the brain stem, cerebellum, and thalamus, with associated lymphocytic meningoencephalitis. Large, intranuclear, eosinophilic inclusions were within regional astrocytes, and the brain lesions were immunohistochemically positive when stained for canine distemper viral antigen. Hematologic and blood chemistry results were consistent with overwhelming systemic infection and starvation. The animal also was antibody-positive for canine distemper virus, feline panleukopenia, and feline coronavirus.

Key words: Canine distemper, Far East, morbillivirus, Russia Siberian tiger.

Morbillivirus infections have been reported in a variety of wild and domestic terrestrial and marine mammals worldwide (Evermann et al., 2001) and could be the single greatest disease threat to populations of susceptible carnivores (Greene and Appel, 2006). Canine distemper virus has caused disease in several species that are common in the Russian Far East, including red fox (*Vulpes vulpes*), wolves (*Canis lupus*), raccoon dogs, (*Nyctereutes procyonoides*), and domestic dogs (*Canis familiaris*; Deem et al., 2000). Interspecies transmission of morbilliviruses has been well documented (Cleveland et al., 2007), but morbidity and mortality rates are species dependant. Epidemics have been reported in several species of felids, including captive tigers and wild African lions (*Panthera leo*; Munson, 2001).

The Siberian tiger (*Panthera tigris altaica*) is highly endangered, with a range restricted to the southern portion of the Russian Far East and northeastern China; the total population is approximately 428–502 individuals (Miquelle et al., 2005). Here, we describe the first recognized morbillivirus infection in a wild free-ranging tiger. On 22 November 2003, an adult tigress (PT-61) entered the village of Pokrovka, Khabarovsky Krai, Russia (46.69°N, 134.03°E). She was in an ambulatory but stuporous condition, non-responsive to stimuli, apparently blind, and unafraid of humans. The tigress remained in or near the village until 26 November 2003, when she was anesthetized and transported 500 km to the town of Terney, where she was confined for evaluation in a temporary holding facility at the headquarters of the Sikhote-Alin Zapovednik (nature reserve). The tigress was in good condition, with normal body weight and fat, a full stomach, and an excellent winter hair coat. Mucous membrane color and capillary refill time were normal. Rectal temperature was 38.5 C, and heart and respiratory rates were normal. She was unresponsive to stimuli, had a fixed stare, appeared blind, and had a clear nasal and ocular discharge. She seemed to have no interest in eating or

TABLE 1. Serologic results from a wild Siberian tiger, Russian Far East.[a]

Infectious agent	Test method (WADDL positive threshold values)	Sample collected 26 November 2003	Sample collected 3 December	Sample collected 14 December
Feline leukemia	ELISA (antigen)[b]	Negative	Negative	Negative
Feline coronavirus[c]	Immunofluorescent antibody (≥1:25)[d]	1:25	1:25	1:25
Feline immunodeficiency	ELISA (antibody)[b]	Neg	Neg	Neg
Canine distemper	Virus neutralization (≥1:4)[e]	Pos @ 1:256	Pos @ 1:256	Pos @ 1:128
Toxoplasmosis	Indirect hemagglutination (≥1:64)[f]	Neg	Neg	Neg
Feline panleukopenia	Immunofluorescent antibody (≥1:25)[g]	Pos @ 1:3,125	Pos @ 1:3,125	Pos @ 1:3,125
Feline calicivirus	Virus neutralization (≥1:4)[d]	Neg	Neg	Neg
Feline herpesvirus	Virus neutralization (≥1:4)[d]	Neg	Neg	Neg

[a] ELISA = enzyme-linked immunosorbent assay; Neg = negative; Pos = positive; WADDL = Washington Animal Disease Diagnostic Laboratory (Washington State University, Pullman, Washington, USA).

[b] FeLV/FIV Snap Test® (IDEXX Laboratories, Westbrook, Maine, USA).

[c] Feline enteric coronavirus/feline infectious peritonitis.

[d] Roelke-Parker et al., 1993.

[e] Guo et al., 1986.

[f] Wampole Laboratory, Princeton, New Jersey, USA.

[g] Evermann et al., 1980.

drinking when food or water was placed in front of her but ate and drank small amounts when food or water was placed into her mouth. Neurological signs included head pressing, ataxia, and intermittent petit and grand mal seizures. Biologists and a local Russian veterinarian were able to force feed the tigress and administer IV fluids and antibiotics daily without anesthesia.

Blood was collected opportunistically; serum samples collected on 26 November 2003 were frozen and shipped to the Pathologist Regional Laboratory (Lewiston, Idaho, USA) and Washington Animal Disease Diagnostic Laboratory (Pullman, Washington, USA). Whole blood samples collected on 4 December and 8 December were analyzed by the Alex Veterinary Clinic (Vladivostok, Russia) and the Terney Regional Hospital (Terney, Russia), respectively.

Hemogram values from the 4 December samples included neutrophilia, with a regenerative left shift, an indication of inflammation and lymphopenia consistent with morbillivirus infections. Hemogram results from the sample collected 8 December included neutropenia with a degenerative left shift and polycythemia, indicating an overwhelming infection and hemoconcentration. Serum chemistry results from 8 December included abnormal values (Quigley et al., 2001; Teare, 2002) for urea nitrogen (8.6 mg/dl; reference range, 20–34 mg/dl), total protein (5.1 gm/dl; reference range, 6.5–7.7 gm/dl), albumin (2.3 gm/dl; reference range, 3.3–4.1 gm/dl), and cholesterol (63 mg/dl; reference range, 177–289 mg/dl), which were all consistent with malnutrition or starvation.

Serum was tested for antibodies to feline coronavirus (FcoV), feline panleukopenia virus (FPLV), canine distemper virus (CDV), feline calicivirus, feline herpesvirus, and *Toxoplasma gondii* (Table 1). Samples also were tested for feline leukemia virus antigen. Antibodies to FcoV, FPLV, and CDV were detected. The possibility that the FcoV- and FPLV-positive results were related to exposure to closely related coronaviruses or parvoviruses or that exposure was associated with consumption of infected prey cannot be discounted (Truyen et al., 1996; Evermann et al., 2001).

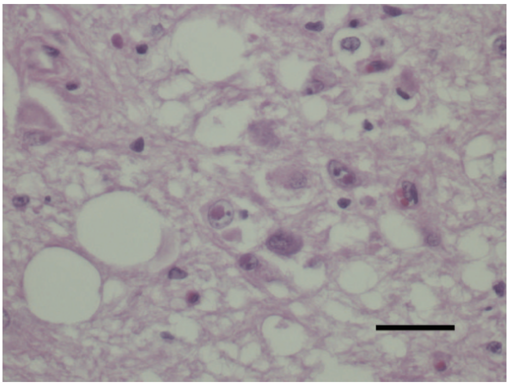

FIGURE 1. Section of the midbrain containing a centrally located glial cell with a large, intranuclear, eosinophilic inclusion body, along with the vacuolated (malacic) brain matter. H&E stain. Original magnification 400×. Scale = 30 μm.

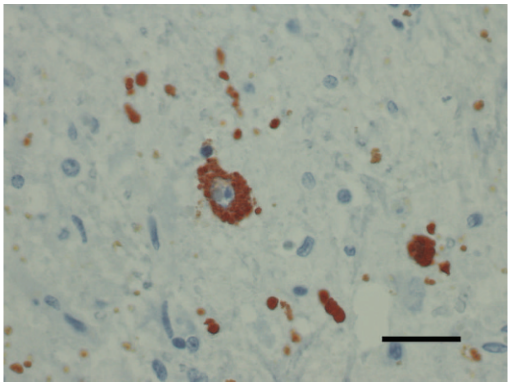

FIGURE 2. Sample from same tissue block of midbrain as in Figure 1, stained immunohistochemically with aminoethylcarbazole for canine distemper viral antigen. Original magnification 600×. Note strong positive staining, especially of the cytoplasm of several glial cells. Scale = 30 μm.

Over the next 6 wk, the tigress' condition deteriorated, and despite intensive supportive care, she died 4 January 2004. Postmortem findings were consistent with emaciation and dehydration. On gross examination, evidence of a focal, acute suppurative bronchopneumonia without identifiable inclusion bodies was found in the lungs. On gross examination of the brain, small focal areas of malacia were noted bilaterally in the areas of the thalamic nuclei and the internal capsule of the brain. Tissue samples from multiple organs and the entire brain were collected, preserved in formalin, and transported to the Washington Animal Disease Diagnostic Laboratory for histopathologic examination. No inclusion bodies or other significant lesions were seen in sections of thyroid gland, larynx, salivary gland, trachea, myocardium, liver, spleen, pancreas, esophagus, stomach, small intestine, colon, kidney, urinary bladder, ovary, or uterus. Histologic lesions in the brain tissue included widespread lymphocytic meningoencephalitis and large malacic areas in the brainstem, cerebellum, and thalamus (Fig. 1). These areas were accompanied by proliferations of glial cells, which often contained brightly eosinophilic, intranuclear inclusion bodies.

Immunohistochemical staining of the brain lesions for canine distemper viral antigen was done with the use of a monoclonal primary antibody to canine distemper virus. This IgG1 antibody was produced in mouse ascites fluid (VMRD Inc., Pullman, Washington, USA; catalog 1C42H11], and appropriate positive and negative control slides were prepared simultaneously. Numerous glial cells and occasional neuron bodies in the malacic areas of the brain stained strongly positive (Fig. 2). No gross or histologic evidence of FPLV or feline coronavirus infection was seen. A diagnosis of morbillivirus infection, consistent with CDV, was made on the basis of the lesions and the staining properties.

In most outbreaks involving morbilliviruses, domestic dogs are implicated as the reservoir (Funk et al., 2001). In the Russian Far East, very few domestic dogs are vaccinated against canine distemper virus, and the disease is common. In addition, because of decreasing habitat, tigers often enter villages, killing and eating dogs; thus, opportunities for transmission are common. It is probable that canine distemper outbreaks in local domestic dog populations in the Russian Far East are the source of morbillivirus in tigers. However, other endemic carnivore populations, including wolves, raccoon dogs, and red foxes, could also serve as reservoirs, and tigers prey on all of these species (Miquelle et al., 1996).

Catastrophic mortality from infectious disease in carnivore populations is more frequently related to morbillivirus infection than any other cause (Young, 1994) and is of particular concern because pandemics involving morbilliviruses have had serious negative effects in numerous carnivore species. Viruses that have multiple hosts are difficult to control and present a challenge in protecting target populations against disease (Evermann et al., 2002). To determine the persistence, ecology, and potential sources of morbilliviruses in the Russian Far East, sampling of domestic and wild carnivore populations is necessary (Murray et al., 1999). If domestic dogs are the primary source of virus for tigers, vaccination of domestic dogs might help decrease the disease threat to wild tigers. Such barrier vaccination programs have been implemented in Africa to minimize crossover of morbilliviruses between domestic and feral dogs and prevent infection in wild lion populations (Cleveland et al., 2007). Vaccination of all dogs throughout the 128,000 km^2 range of Siberian tigers would be extremely difficult, controversial, costly, and labor intensive, but it might be of significance to the long-term health of the tiger population.

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