### AEMV FORUM

## TWO CASES OF SYSTEMIC CORONAVIRUS-ASSOCIATED DISEASE RESEMBLING FELINE INFECTIOUS PERITONITIS IN DOMESTIC FERRETS IN JAPAN



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#### Abstract

A systemic disease of domestic ferrets characterized by pyogranulomatous inflammation was first recognized in Europe and the United States in 2002. The disease closely resembled feline infectious peritonitis and subsequently has been shown to be associated with ferret systemic coronavirus (FRSCV). A definitive laboratory diagnosis of this disease is typically based on a combination of immunohistochemistry (IHC) and reverse-transcriptase polymerase chain reaction tests to detect FRSCV in granulomatous lesions. In 2010, this feline infectious peritonitis–like disease was first identified in a laboratory ferret in Japan, and laboratory confirmation of the clinical diagnosis was limited to IHC. This report describes 2 cases of systemic coronavirus-associated disease in ferrets presented to Japanese veterinary hospitals. Both presented with pyogranulomatous inflammation in the abdominal cavity, and both cases tested positive for coronavirus antigen by IHC. In 1 case, for which unfixed tissues were available, FRSCV RNA was detected by reverse-transcriptase polymerase chain reaction in the affected tissues. Copyright 2014 Published by Elsevier Inc.

Key words: coronavirus disease; ferret; FIP; Japan; pyogranulomatous inflammation

eline infectious peritonitis (FIP) is a fatal, multisystemic, immune-mediated disease of cats caused by a feline coronavirus mutant, generally considered to arise spontaneously from subclinical low-pathogenic or nonpathogenic feline enteric coronavirus.<sup>1</sup> Recently, another hypothesis was presented,<sup>2</sup> which states that genetically distinct avirulent and virulent forms of feline coronavirus cocirculate in natural cat populations. In ferrets, epizootic catarrhal enteritis, caused by the ferret enteric coronavirus (FRECV), is widely recognized.<sup>3,4</sup> More recently, a new ferret systemic coronavirus (FRSCV)-associated ferret disease, closely resembling the granulomatous or dry form of FIP, was reported in the United States, Europe, and Japan.<sup>5-7</sup> Although it is unknown whether FRSCV and FRECV are genetically distinct coronaviruses of ferrets, 3 geographically distinct systemic ferret coronavirus strains were found to share a conserved spike (S) gene genotype that was distinguishable from that of 3 independent enteric coronavirus strains.<sup>8</sup> Based on this finding, 2 S gene genotype-specific reverse-transcriptase polymerase chain reaction (RT-PCR) assays had been developed that can potentially differentiate FRSCV from FRECV.<sup>8</sup> The authors were recently involved with 2 ferret cases that were presented to private veterinary practices with clinical signs consistent with FIP-like disease. All confirmatory testing by immunohistochemistry (IHC) and RT-PCR was conducted at the Diagnostic Center for Population and Animal Health at Michigan State University (Lansing, MI USA) using the previously described methods.<sup>3,4,8</sup>

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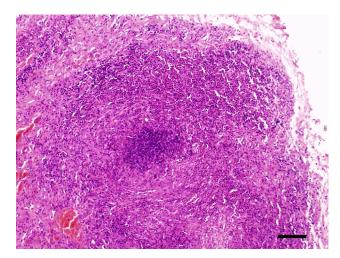
#### CASE 1

A 15-month-old castrated male domestic ferret weighing 840 g was admitted to the Ouji Pet Clinic (Tokyo, Japan) for evaluation of significant weight loss. The ferret also exhibited alopecia in the tail region, anorexia, ataxia, and paresis of all 4 limbs. Blood glucose concentration was within reference ranges (78 mg/dL, reference limits: 69 to 139 mg/ dL). During an ultrasonographic examination of the abdominal cavity, a mass (10 mm in diameter) was identified. Enrofloxacin (5 mg/kg orally, twice a day, Baytril; Bayer HealthCare, Manheim, Germany), prednisolone (1 mg/kg POq 12 h) (Predonine Tablets 5 mg, Shionogi & Co., Ltd., Osaka, Japan), and B-complex vitamins were prescribed. An improvement in the ferret's appetite was observed during the 12-week course of treatment. The ferret died 135 days after the initial visit. At necropsy, white nodules, ranging from 5 to 10 mm in diameter and dispersed over the serosal surface of all lung lobes, were detected. Enlargement of the adrenal glands and granulomatous lesions around the enlarged mesenteric lymph node were also present. There were no remarkable gross changes in the brain. Histopathologically, multifocal granulomatous inflammatory lesions, consisting of macrophages, neutrophils, and lymphocytes, were observed in the lung, adrenal gland, intestinal tract (Fig. 1), mesenteric lymph node, and meninges of the brain. Immunohistochemical analyses of tissues were performed using the antialphacoronavirus monoclonal antibody, FCV3-70a, as previously described.<sup>3,9</sup> Immunohistochemical staining demonstrated the presence of coronavirus antigens in the mesentery. RT-PCR testing was not performed because unfixed tissues were not available. The formalin-fixed tissue samples for this case had undergone overfixation (>48 hours in formalin) and were no longer suitable for RT-PCR testing. Mycobacteriosis was not observed with Ziehl-Neelsen or Periodic acid-Schiff stains.

#### CASE 2 \_

A 20-month-old spayed female ferret, weighing 540 g, was presented for evaluation of significant weight loss and anorexia. A physical examination revealed the presence of an abdominal mass, which was 30 mm in diameter. Abnormal results from the complete blood count and plasma biochemistry panel indicated leukocytosis (14,900 per  $\mu$ L, reference limits: 3500 to 7000 per  $\mu$ L), hyperglycemia (221 mg/dL, reference limits: 69 to

139 mg/dL), elevated aspartate amino transferase (323 U/L, reference limits: 38 to 89 U/L), hyperproteinemia (12.0 g/dL, reference limits: 5.1 to 7.8 g/dL), and hyperglobulinemia (60.2%, A/G = 0.28). Aleutian disease (AD) virus (ADV)specific antibody titers were negative. An abdominal ultrasonography examination revealed a hypoechoic abdominal mass measuring  $33.2 \times$ 19.4 mm<sup>2</sup>, with significant blood flow. An ultrasound-guided fine-needle aspiration biopsy of the mass was collected. The results revealed pyogranulomatous inflammation, consisting of macrophages, neutrophils, and lymphocytes. On day 1, enrofloxacin (5 mg/kg, orally, twice a day) and prednisolone (1 mg/kg, orally, once a day) were prescribed. No improvement was noted, and an exploratory laparotomy was performed on day 7. Granulomatous lesions of the intestinal tract, mesentery, mesenteric lymph node, spleen, and retroperitoneum were found. Histopathologically, multifocal granulomatous inflammatory lesions consisting of macrophages, neutrophils, and lymphocytes were observed in the mesentery and greater omentum. Mycobacteriosis was not detected with Ziehl-Neelsen or Periodic acid-Schiff stains. After surgery, prednisolone was increased to 2 mg/kg by mouth once a day and enrofloxacin increased to 10 mg/kg by mouth once a day. On day 24, the ferret showed an improved appetite, and an abdominal ultrasonography examination revealed a reduced abdominal mass, now 22.2  $\times$  $12.0 \text{ mm}^2$ . On day 50, the mass could not be detected by palpation. Although the animal's overall condition continued to decline 150 days



**FIGURE 1.** Small intestine of a ferret with confirmed ferret systemic coronavirus infection. Note multifocal granulomatous inflammation consisting of macrophages, neutrophils, and lymphocytes. Bar =  $300 \,\mu$ m. H&E stain. H&E, hematoxylin and eosin.

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