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SYSTEMIC CALICIVIRUS EPIDEMIC IN CAPTIVE EXOTIC FELIDS

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Abstract: A 5-day-old, mother-raised, Amur tiger cub (*Panthera tigris altaica*) presented with tongue ulcerations. Identical lesions appeared and progressed to sloughing of the tongue in the three littermates of this cub the following day. The lesions progressed in all cubs to include sloughing of the carpal, tarsal, metacarpal, and metatarsal foot pad epithelium. Oral ulcerations were also noted in adult African lions (*Panthera leo*) and Amur tigers (*Panthera tigris altaica*), but not in two adult snow leopards (*Panthera uncia*) housed in the same building. All adult cats had been previously vaccinated for common feline diseases including feline calicivirus (FCV). Detection of FCV RNA in oral secretions by a real-time reverse transcription polymerase chain reaction assay (RRT-PCR) confirmed FCV infection in the tiger cubs and one lion. A male lion and a male tiger cub died during the disease outbreak. RRT-PCR confirmed FCV in multiple tissues in both of these animals. A stray cat live-trapped outside the feline building during the epidemic was found to be positive for FCV by virus isolation and was thought to be the source of infection.

Key words: African lion, Amur tiger, captive felid, feline calicivirus, systemic calicivirus.

INTRODUCTION

Feline calicivirus (FCV) infection is commonly associated with upper respiratory tract disease in domestic felines and is characterized by fever, rhinitis, oral ulcers, and pneumonia.[10] Strains of FCV resulting in severe systemic forms of FCV infection have been recognized, particularly as outbreaks in catteries or humane shelters.[9,18] These virulent systemic strains of FCV have been associated with additional clinical signs, such as cutaneous ulcers, subcutaneous edema, and alopecia and with histopathologic findings including pancreatic, hepatic, and splenic necrosis. Virulent FCV has caused mortality rates as high as 60% in domestic cats.[9,18] Reports of FCV infections in exotic felids are rare, and are limited to the respiratory form or to detection of FCV antibodies from natural infection.[8,11,16,23] This case series documents an outbreak of virulent, systemic FCV infection in captive exotic felids associated with mortality.

CASE REPORT

A 5-day-old female, mother-raised Amur tiger cub (*Panthera tigris altaica*) presented with mild dehydration and a focal ulcer on the right lateral tongue. The cub and its three littermates were housed in a holding area of a feline building that also housed a male and female each of the following species, African lion (*Panthera leo*), Amur tiger (*Panthera tigris altaica*), and snow leopard (*Panthera uncia*). The following day, the cub's three littermates, two males and a female, presented with similar oral ulcerations and mild dehydration. The tiger cubs were separated from their mother once a day to provide symptomatic and supportive care for their lingual ulcerations, to monitor weight gain, and to evaluate nutritional status. The extent and severity of lingual lesions progressed in all four tiger cubs to include ulcerations at the base, and necrotic areas along the rostral portion of the tongue (Fig. 1). All cubs sloughed a full-thickness portion of their tongue starting at 5 days post-outbreak. Their mother presented with focal ulceration of her nasal epithelium at the same time. Oral secretions were collected from all four tiger cubs on day 5 for virology studies (Table 1). The male tiger that sired the cubs, but had no contact with them, presented with lingual ulcerations along the right lateral surface of the tongue on day 7. Additionally on day 7, the smallest female tiger cub was found to be dehydrated, hypothermic, and unable or unwilling to suckle, with necrosis and sloughing of the distal portion of the tongue. It was removed from the mother to be hand-raised. Foot pad ulcerations were also noted in the remaining three cubs. The smallest male cub was found to be weak, dehydrated, and hypothermic and was pulled for hand-rearing on day 8. At 9 days, when the cubs were 15 days old, the other male tiger cub, still being raised by the mother, was found dead in the holding den and the remaining female cub was removed from the moth-

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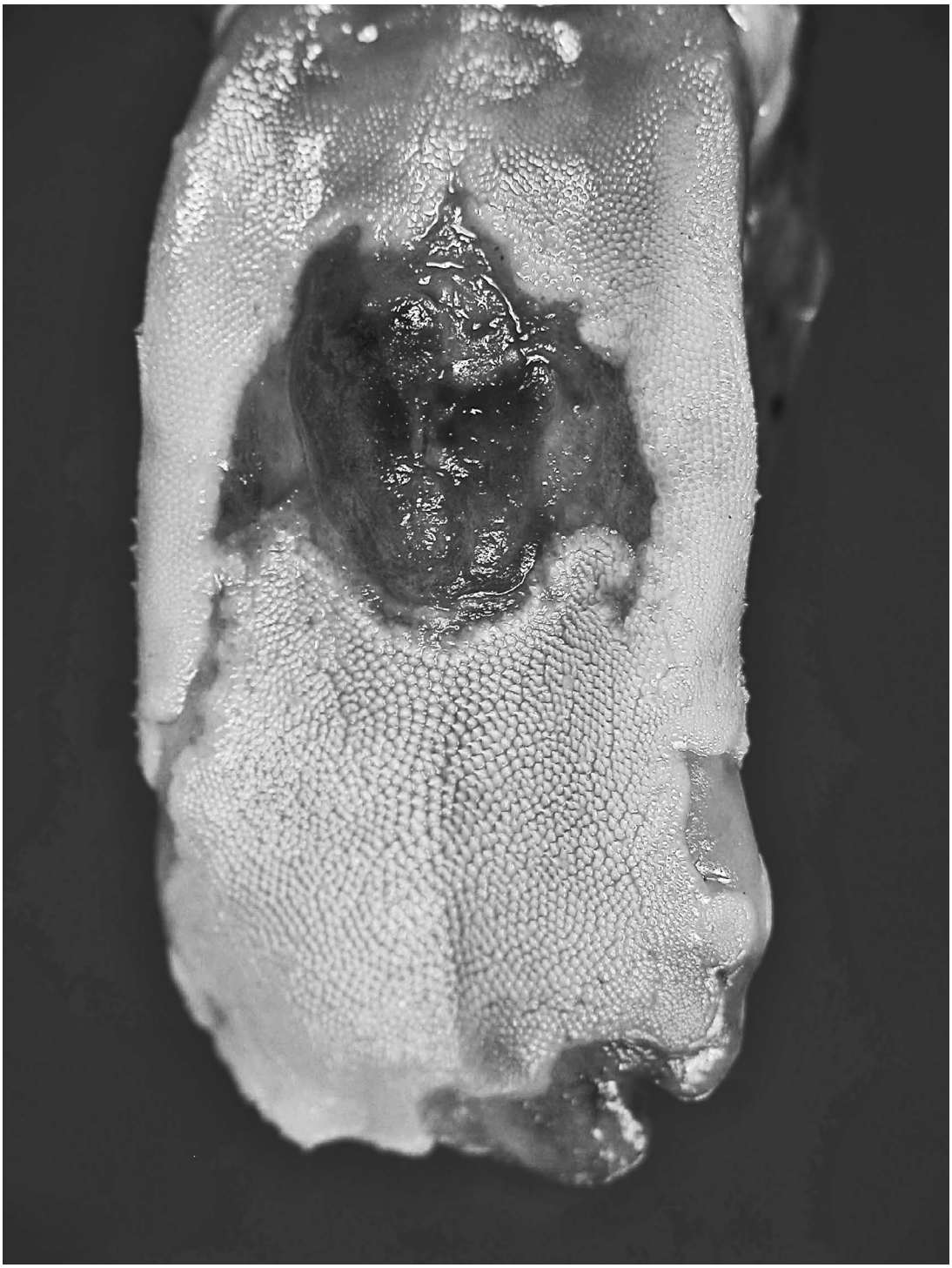

Figure 1. Tongue from tiger cub that died on day 12 of a virulent systemic feline calicivirus infection. Note the necrosis and sloughed tissue from the distal tip of the tongue and the mucosal ulceration of the base of the tongue.

Table 1. Serum neutralization titers, day clinical signs were observed, real-time reverse transcription polymerase chain reaction assay (RRT-PCR) results, and virus isolation results for feline calicivirus in exotic felids housed in feline house that experienced a calicivirus epidemic. Days are reported in relation to day 1, the first day that clinical signs were noted in the first case of the outbreak.

Species of animal	Sex of animal	Day that serum sample was collected for calicivirus titer testing	Calicivirus titer prior to and after outbreak	Day that vaccination was given to cats for calicivirus
African lion 1	Male	−128	<4	−489
African lion 2	Female	−393	64	−393
Amur tiger 1	Male	−16	<4	−16
Amur tiger 1	Male	249	128	
Amur tiger 2	Female	−183	<4	−421
Amur tiger 2	Female	188	256	
Amur tiger cub 1	Female			
Amur tiger cub 1	Female			
Amur tiger cub 2	Male			
Amur tiger cub 2	Male			
Amur tiger cub 3	Female			
Amur tiger cub 3	Female			
Amur tiger cub 4	Male			
Snow leopard 1	Female	−63	32	−63
Snow leopard 2	Male	68	<4	7
Stray cat	Male			

er for hand-rearing. Procaine penicillin G (Phoenix, St. Joseph, Missouri 64503, USA; 20,000 IU/kg i.m., s.i.d. for 10 days) was given to the cubs once they were removed from the mother and could be treated reliably.

The foot pad ulcerations of the surviving tiger cubs were completely healed after 24 days of symptomatic care with topical application of an ointment containing zinc oxide, cod liver oil, lanolin, and petrolatum (Desitin®, Pfizer, Morris Plains, NJ 07950 USA) four to five times a day. The tongue ulcerations in the tiger cubs were completely healed 55 days after the initial onset of clinical signs. The lingual ulcerations in the adult male tiger were resolving at day 22.

The dead cub weighed 1.4 kg, and was markedly thin. There were irregular ulcers, ranging in size from 2 mm to 1 cm, on all digital metacarpal, carpal, and metatarsal footpads (Fig. 2). The carpal pad epithelium was completely denuded. An irregular ulcer covered the dorsal surface of the distal tongue with a 1–2-mm-wide linear ulcer extending along the right dorsal lateral tongue surface to the tip. The edge of the left side of the tongue was completely ulcerated. Grossly, there was an absence of adipose stores. Histologically, the lesions were consistent with an ulcerative and necrotizing glossitis and an ulcerative pododermatitis. There was evidence of a secondary bacterial infection in the affected tissues. Real-time reverse transcription polymerase chain reaction assay (RRT-PCR) for FCV RNA was positive in the spleen, tongue, oral cavity, bronchial lymph node, esophagus, thyroid gland, synovial membrane, and foot pad.[17,20] Tissues were negative for feline panleukopenia virus (FPL) and feline herpes virus (FHV)–1 by RRT-PCR.

On day 6 of the outbreak, a 16-yr-old male African lion that had a splenectomy and was undergoing chemotherapy for lymphoma for 504 days, presented with four circular ulcerations on the medial aspect of the tongue and left forelimb lameness.[7] On day 13, the male lion was noted to be sensitive on all limbs. Eighteen days post-outbreak, the female lion, who was housed with the male lion, presented with a focal ulceration on the medial aspect of the dorsal surface of her tongue. The female's lingual ulcerations were resolving on day 22. On Day 19, the male lion was found dead in his holding den with black tarry diarrhea. Gross and histological findings indicated the presence of severe anemia, including diffusely pale tissues and acute centrilobular hepatocellular necrosis. There were histologic findings consistent with the lymphoma as described previously.[7] FCV RNA was present via RRT-PCR in the following tissues: synovial membrane, liver, lung, intestine, tongue, lymph node, adrenal gland, esophagus, thyroid gland, and parathyroid gland.

An asymptomatic stray cat was live-trapped just

Table 1. Extended

Day that clinical signs were observed	Day that oral swab sample was collected for calicivirus RRT-PCR	Calicivirus RRT-PCR results	Day that sample was collected for calicivirus virus isolation	Calicivirus virus isolation results
6	13	Positive	19	Positive
18				
7	37	Negative		
5				
1	13	Positive	5	Positive
	35	Negative		
2	13	Positive	5	Positive
	35	Negative		
2	13	Positive	5	Positive
	35	Negative		
2	9	Positive	5	Positive
			5	Positive

outside the feline building on day 5. Oral secretions and a blood sample were collected from the domestic cat for viral serologies and isolation attempts (Table 1).

Strict quarantine was placed on the entire feline building at the time of the epidemic to minimize spread of FCV to other felids in the zoo. No animals were moved into or out of the building, and entrance of people into the feline holding area was minimized. Quaternary ammonia was used for footbaths, cleaning the cages, and disinfecting instruments. Instruments were limited to use for each species only. No other zoo felids exhibited clinical signs or visual evidence of infection. Quarantine was maintained on the building for 1 mo past the resolution of all clinical signs in the affected animals. Cleaning protocols were changed to clean each exhibit and holding area separately for each feline species and enrichment items were no longer shared between species.

Eight days after the start of the outbreak, all adult felids in the building were vaccinated by projectile dart for FCV, FPL, FHV, and feline coronavirus (FCoV) (Fel-O-Vax IV, Fort Dodge, Fort Dodge, Iowa 50501 USA; 1 ml i.m.).

Viral studies

Oral swab samples obtained from the tiger cubs on day 5 were positive by virus isolation (VI) for FCV (Table 1). On day 13, oral swab samples from all of the tiger cubs were positive by RRT-PCR for FCV but repeat samples at day 35 were negative (Table 1). On day 41, the FCV virus neutralization (VN) titers of the two female and male tiger cubs were 2,048, >4,096, and >4,096, respectively.

A sample taken from the lion while he had active oral lesions was positive for FCV by RRT-PCR (Table 1). Samples taken from the female lion and adult female tiger after the oral lesions resolved were negative for FCV by RRT-PCR.

The stray cat was also positive for FCV by VI on day 5 (Table 1). The stray cat was negative for feline leukemia virus antigen and feline immunodeficiency virus antibody by enzyme-linked immunosorbant assay.

Serum samples that were available from all cats in the holding facility prior to the outbreak and after the outbreak were submitted for FCV VN testing (Table 1).

DISCUSSION

Infection with FCV has been reported in lions and tigers.[11,23] In neither of these reports, however, was severe morbidity or mortality observed.[11,23] In the present report, FCV infection spread to numerous big cats, as diagnosed through RRT-PCR, systemically in tissues including tongue and foot pad ulcerations in the tiger cubs, and tongue ulcerations and viral arthropathy in the previously vaccinated, immunocompromised lion. The effects in the im-

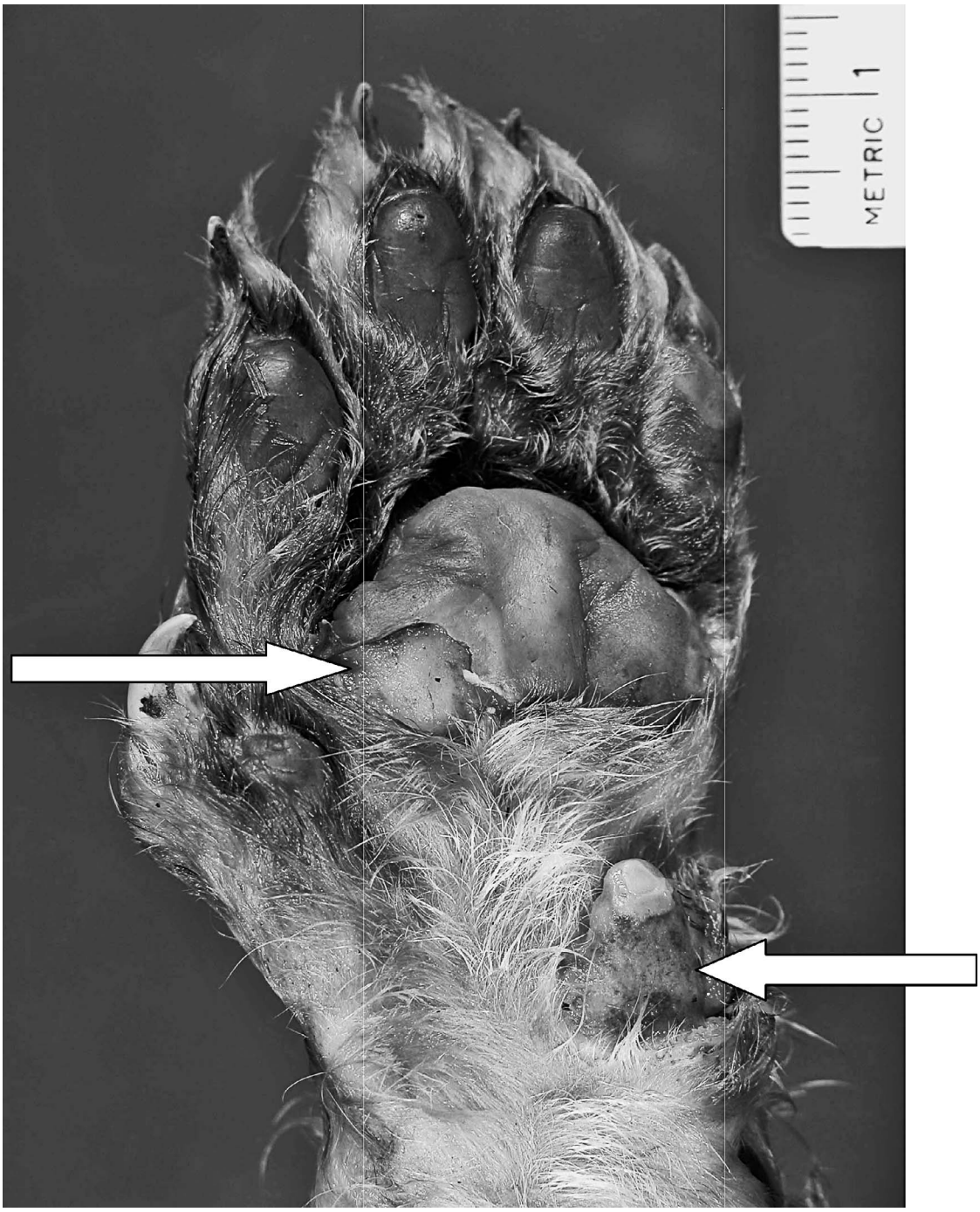

Figure 2. Paw from tiger cub that died on day 12 of a virulent systemic feline calicivirus infection. Ulcerations present on the carpal and metacarpal foot pads are indicated by arrows.

munocompromised lion may have been exaggerated because of his having been splenectomized and undergoing chemotherapy treatments. The nature, severity, and the extent of clinical signs associated with FCV infection in this collection of exotic cats are similar to those reported in domestic cats with the virulent systemic strains of FCV.[9,18] Isolating FCV from tissues such as the synovial membrane,

foot pad, spleen, tongue, oral cavity, and liver, among other tissues, is indicative of a systemic calicivirus infection. It is difficult to determine whether the disease manifestations observed in this outbreak reflect responses to a virulent systemic FCV strain, or represent infection with a conventional FCV strain in immunocompromised hosts, such as the neonatal tiger cubs and the lion being treated for lymphoma. Vaccination with killed FCV vaccines has historically been practiced in zoological institutions in an attempt to prevent or minimize clinical disease induced by inadvertent exposure of their exotic felid collections. All adult cats had been vaccinated with a killed polyvalent vaccine, containing FCV, FPL, FHV, and FCoV (Fel-O-Vax IV, Fort Dodge) within 25 mo prior to the onset of the outbreak. Vaccination with modified live FCV vaccines has been shown to provide protection for up to 3 to 4 yr in domestic cats.[12,13,19] Serologic testing and challenge trials have not been performed in exotic felids vaccinated with the killed FCV vaccine product that is currently recommended by the veterinary advisors for various species survival plans.[1,21] Booster vaccinations in zoological institutions may occur annually or at longer intervals depending on the institution. The male lion had not been vaccinated for 14 mo because of concerns relating to his lymphoma treatment.[7] The snow leopards did not develop any clinical signs of FCV nor did they have detectable levels of FCV-neutralizing antibodies, despite prior FCV vaccination and being in the same building as the other affected cats. All other cats housed in the facility were affected. The susceptibility to FCV disease and lack of neutralizing antibody levels in these cats despite routine vaccination raises the question of vaccine efficacy and or duration of immunity induced by the FCV vaccination protocols currently recommended.

Exposure and spread of virulent systemic FCV in domestic cats occurs in situations where numerous felines are concentrated in confined areas, such as veterinary hospitals or shelters.[9,10,15,18] Prior FCV vaccination may not always be protective in these situations, because the virulent systemic FCV strain responsible for the outbreak may be genetically and serologically distinct from strains used in commercial FCV vaccines.[10] The housing of six adult felids in a feline building at this zoological institution is not considered equivalent to housing a large number of cats. The potential routes of transmission between cages could have been from zoo staff cleaning the lion and tiger exhibits or a domestic cat entering the lion and tiger outdoor exhibits. The snow leopard exhibit had a separate entrance and holding area that was cleaned separately from the lion and tiger exhibits. The lion and tiger exhibits and their holding areas were cleaned simultaneously by entering one exhibit or holding area through the adjacent area. Enrichment items were also shared between the lion and tiger exhibits and holding areas, whereas the snow leopards received their own enrichment items. The physical separation of the snow leopard facility and care from the lions and tigers may explain the lack of transmission of the infection to these cats.

The source of FCV infection in this series of exotic cats is hypothesized to be via the infected but asymptomatic domestic feline described in this report. After recovery from acute illness, FCV-infected cats may continue to shed the virus in saliva without clinical signs of infection for months, or possibly years.[14,22] The domestic cat live-trapped in the vicinity of the exotic felid exhibit was found to be positive for FCV by VI. A portion of the capsid gene of the FCV isolates obtained from one of the tiger cubs, the male lion, and the stray cat were sequenced and found to be identical (Maes, pers. comm.).[20]

Although contact between domestic cats and exotic felines is usually minimal, at the time of the epidemic, the outside exhibits were empty at night and a domestic cat could have entered the outside portion of the exhibits. The mesh size of the lion and tiger exhibits was 10 by 15 cm, which could allow access by most domestic cats. The mesh size of the snow leopard exhibit was 5 by 5 cm, which would exclude most domestic cats. FCV can survive in the environment in a dried state at room temperature (20°C) for up to 28 days.[3] It is conceivable that the lions and tigers were exposed in their outside exhibits to FCV introduced by the domestic cat.

The spread of FCV to the other felines likely had numerous contributing factors. The incubation period of FCV can be up to 14 days, so the female tiger may have been exposed to FCV prior to being denned up 1 wk before the birth of the cubs, and then later infected them through horizontal transmission. Additionally, the virus may have been transferred to the other felines via fomites, particularly because it can be difficult to inactivate the virus.[2,4,5]

The mortality experienced during this outbreak was indirectly related to FCV. The neonatal tiger cub that died was unable to suckle adequately because of the tongue ulcerations and likely died from dehydration, emaciation, and hypoglycemia. The death of the male lion was mostly because of his chemotherapy treatment, but it is likely that FCV caused an added stress to this already immunocom-

promised individual. All other immunocompetent adults that developed clinical signs in association with FCV infection recovered uneventfully. The three remaining tiger cubs also recovered after extensive supportive care. The tongue and footpad lesions resolved with minimal scarring in the tiger cubs.

Zoological institutions may not consider themselves at risk for outbreaks of virulent FCV strains, because of their existing vaccination protocols and limitation of exposure to domestic felines. Although those same practices were employed at this zoological institution, they did not prevent morbidity and mortality associated with an outbreak of virulent FCV among this group of exotic felids. By nature, FCV is capable of rapid mutation and is characterized by a high level of antigenic and pathogenic variation.[6] Under these circumstances, vaccine-induced cross-protection may be compromised. Additional studies are in progress to evaluate the efficacy of killed virus vaccines and vaccination protocols used in exotic zoo felids. Zoological institutions should be vigilant in their efforts to exclude domestic animals, and be aware that vaccination of their animals may not be protective against a similar outbreak of virulent FCV.

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