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Detection of feline coronavirus in captive Felidae in the USA

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Abstract. Feline coronavirus (FCoV) is an important pathogen of domestic and nondomestic Felidae. Investigation into the prevalence of FCoV in exotic Felidae has relied primarily on serology. The usefulness of genetic detection of FCoV using reverse transcription and nested polymerase chain reaction (RT/nPCR) for viral screening was investigated. Seventy-five biologic samples, primarily feces, from captive felids from 11 institutions were tested using PCR. Serum samples collected from all but 12 of these animals were tested for antibodies to type I and type II FCoV by indirect immunofluorescence. Twenty-four animals were positive using RT/nPCR for virus. Twenty-nine animals were seropositive to type I and/or type II FCoV. From serologic data, infection with a virus antigenically related to FCoV type I occurred most commonly. Serology did not correlate with virus shedding because 13 animals were seronegative to FCoV type I and II but positive using RT/nPCR for virus. Conversely, 20 animals were seropositive but negative using RT/nPCR for FCoV. Some of the populations in which virus was detected had experienced health problems, including feline infectious peritonitis (FIP), necrotizing colitis, and mild enteritis. In addition to its role in FIP, this virus may play a role in gastrointestinal diseases of infected animals. This study demonstrates that FCoV is a significant infectious agent of captive felids because over half of the animals tested were positive by viral genetic detection, serology, or both. Dependence upon one method for detection of infection is unreliable.

Infectious diseases are potentially devastating to wildlife conservation. In particular, large carnivore populations are susceptible to serious consequences because they are already threatened by restricted ranges, habitat destruction, and overexploitation of themselves and their prey.[9] In addition, extenuating circumstances such as inbreeding, stress, or malnutrition can worsen the outcome of infection with a pathogen.[9] Feline coronavirus is a contagious and serious pathogen of Felidae. It is associated with mild to severe enteritis and is the etiologic agent of feline infectious peritonitis (FIP), a fatal disease.[2,6] In domestic cat populations, FIP is a sporadic disease though the virus is ubiquitous.[10] In contrast, outbreaks of FIP have been reported in several nondomestic species.[1,3,13] In addition, feline coronavirus (FCoV) enteritis has resulted in mild to severe chronic diarrhea in several felid species and has been associated with vague signs of disease including weight loss, depression, and inappetence.[3,8] Control of this pathogen is complicated by the occurrence of persistent infections, with carriers serving as an important source of the virus in felid populations.[8] The vulnerability of exotic felid populations and the significant exposure rate emphasize the need for effective screening methods. The usefulness of FCoV genomic detection using reverse transcription and nested polymerase chain reaction (RT/nPCR) was investigated. Virus genetic detection was compared with serology in screening exotic felids for FCoV infection.

Biological samples were collected from 75 captive felids from 11 zoologic institutions in the USA for FCoV genetic detection. Species of Felidae tested included cheetah, snow leopard, African leopard, serval, Bengal tiger, Siberian tiger, mountain lion, African lion, lynx, ocelot, jaguar, and bobcat. Fecal samples were used for genetic detection, from all but 6 animals. For these animals, no feces were submitted, and only blood ($n = 5$) or blood and effusion ($n = 1$) were provided for testing using RT/nPCR. Serum or plasma was provided for serologic testing from all but 12 animals for FCoV-specific antibodies.

Total RNA was extracted from the specimens using Trizol LS according to the manufacturer's directions.[a] The RNA was taken to reverse transcription using Moloney murine leukemia virus reverse transcriptase according to the manufacturer's recommendations.[a] The downstream external primer was used for first strand synthesis as described previously.[7] Polymerase chain reaction was done using Ex-*Taq* polymerase as described previously with the upstream external primer.[b,7] This was followed by nPCR using internal primers.[7,8] For the majority of cheetah samples, primers used encompassed the entire 7a7b open reading frame (ORF), the 3′-most ORF of the FCoV genome ($n = 33$).[7,8] This region has been associated with virulence of FCoV and may play a role in disease production; additionally, mutations are known to occur in this region.[11,12] To investigate the occurrence of mutations in this region and identify correlates with virulence, the 7a7b region of the FCoVs infecting cheetahs were amplified and cloned and nucleotide sequences analyzed (data not shown). Samples from 10 cheetahs and other species were tested using primers encompassing the untranslated region because this region is highly conserved among group I coronaviruses ($n = 42$).[4] Sensitivity and specificity determination were described previously.[7] Products were evaluated by electrophoresis on 1% agarose gels.

Detection of FCoV-specific antibodies was done for all

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Table 1. Feline Coronavirus RT/nPCR—positive animals.

Institute	Species	Population health status*	FCoV-specific antibody†	
			Type I	Type II
A	Cheetah	Healthy	<5	<5
B	Bobcat	NA‡	<5	<5
B	Bobcat	NA	<5	<5
C	Cheetah	FIP	>640	<5
C	Cheetah	Healthy	<5	<5
D	Cheetah	Healthy	<5	<5
F	Siberian tiger	Healthy	<5	<5
H	Cheetah	Chronic diarrhea	<5	<5
H	Lynx	Healthy	<5	<5
H	African lion	NA	<5	<5
H	Jaguar	Healthy	<5	<5
H	Jaguar	Healthy	<5	20
I	Cheetah	Weight loss, decreased appetite	160	<5
J	Snow leopard	Healthy	<5	<5
J	Cheetah	Healthy	<5	<5
K	Cheetah	Necrotizing colitis/intermittent diarrhea	320	<5
K	Cheetah	Necrotizing colitis/intermittent diarrhea	10	<5
K	Cheetah	Necrotizing colitis/intermittent diarrhea	20	<5
K	Cheetah	Necrotizing colitis/intermittent diarrhea	>640	<5
K	Cheetah	Necrotizing colitis/intermittent diarrhea	>640	640
K	Cheetah	Necrotizing colitis/intermittent diarrhea	5	<5
K	Cheetah	Necrotizing colitis/intermittent diarrhea	80	10
K	Cheetah	Necrotizing colitis/intermittent diarrhea	10	<5
K	Cheetah	Necrotizing colitis/intermittent diarrhea	<5	<5

* Health status of subject animal and/or contact animals.
† Expressed as reciprocal of highest dilution resulting in fluorescence.
‡ NA = Not available.

animals from which serum or plasma were provided (n = 63). Serology was done by indirect immunofluorescence as previously described using FCoV type I (UCD1) and type II (WSU 1143) as the capture antigens.[c,7] Antibody was detected with rabbit anti-feline immunoglobulin G conjugated to fluorescein isothiocyanate.[d] The titer was reported as the reciprocal of the highest dilution in which fluorescence was observed. Antibody titers of <5 were considered negative.

Twenty-four of the 75 animals tested (32%) were positive for FCoV using RT/nPCR (Table 1). These animals were from 9 of the 11 institutions (82%) in the study. Twenty-nine of 63 animals tested by serology (46%) were seropositive to type I and/or type II FCoV and were from all 11 institutions in the study. Thirty-one animals (41%) showed no evidence of virus infection by virus detection alone (n = 10; serology not done) or virus detection and serology (n = 21). Of the RT/nPCR-positive and seropositive animals (n = 11), 10 had higher antibody titers to type I than to type II FCoV, and in fact, 8 of these were seronegative to type II. Interestingly, 13 animals were seronegative to FCoV type I and type II but were positive for FCoV shedding using RT/nPCR (agreement of 54%; Table 1).

Antibody levels in seropositive animals ranged from 5>640. Eleven of the 63 animals tested (17%) were seropositive to type I only, and 13 (21%) were seropositive to type II only. Only 5 animals (8%) had antibody detectable to type I and type II FCoV. Of these, 4 had higher antibody levels to type I than type II FCoV. Thus, for all seropositive animals (n = 29), 15 had higher antibody levels to type I than type II FCoV. Thus, the infecting virus was most often antigenically related to FCoV type I, although by a small margin, as indicated by serology. This is consistent with the findings in domestic cat populations where FCoV type I is more prevalent.[5] The disparity in virus detection compared with serology was evident here as well because 20 animals were seropositive to at least 1 type of FCoV but were negative for virus shedding using RT/nPCR (agreement of 31%; Table 2).

Populations testing positive from at least 4 institutions (36%) had experienced disease ranging from mild enteritis to fatal FIP, and all the affected groups were cheetahs. Health history of the animals was not available from 3 institutions. Diseases and abnormalities reported included mild intermittent to chronic diarrhea, weight loss, decreased appetite, necrotizing colitis, and FIP. Although FCoV may not be the sole agent of disease in all cases, we speculate that it is at least a contributing factor to the illnesses. Cheetahs are known to be highly susceptible to disease after infection with FCoV, perhaps because of their lack of genetic heterogeneity as a species.[1]

Detection of FCoV in feces of seronegative animals is a cause of concern because many institutions screen for infection before transport or introduction to a population by serology only. The disparity in virus detection may be explained by the presence of low levels of virus in the gastrointestinal tract, insufficient to induce a systemic antibody response but sufficient to be detected using nPCR, or by early stages of infection before development of an antibody

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Table 2. Feline coronavirus RT/nPCR—negative and seropositive animals.

Institute	Species	FCoV-specific antibody*	
		Type I	Type II
A	Cheetah	>640	<5
A	African lion	20	10
C	Cheetah	320	40
E	Cheetah	<5	20
E	Cheetah	<5	10
E	Cheetah	<5	10
E	Cheetah	<5	10
F	Bengal tiger	160	20
F	Bengal tiger	80	80
F	Bengal tiger	<5	80
G	Cheetah	<5	10
H	Cheetah	20	<5
H	Lynx	<5	10
I	Snow leopard	<5	40
I	African leopard	<5	20
I	African leopard	<5	40
J	African leopard	<5	40
J	Snow leopard	<5	320
K	Cheetah	40	<5
K	Cheetah	10	<5

* Expressed as reciprocal of highest dilution resulting in fluorescence.

response. Alternatively, the FCoV infecting these animals may be antigenically distinct from those used in the serologic assay. It was evident from serologic testing with FCoV types I and II that antigenic variation had a dramatic effect on the results observed because markedly disparate results were obtained often depending upon the virus type used. A similar situation was observed in a previous study in which virus was detected in feces by using electron microscopy, but serology on the same animals was negative for FCoV-specific antibody.[3] The authors speculated that there may be several immunologically distinct strains of coronaviruses.

This investigation revealed that over half of the felids tested were positive for FCoV infection by either genetic detection or serology and included 8 different species from all submitting institutions. A total of 44 of the 75 animals tested (59%) were seropositive or RT/nPCR-positive (or both) for FCoV, indicating current or past infection. Of animals tested using both RT/nPCR and serology (n = 63), 44 animals were positive by one or both assays (70%). Of these 63 animals, 24 (38%) were positive using RT/nPCR and 29 (46%) were positive by serology. Thus, we recommend testing an animal at least twice at 30-day intervals for virus shedding using RT/nPCR along with serology before introduction to a population where FCoV infection is a concern.

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Sources and manufacturers

a. Gibco BRL, Baltimore, MD.
b. Intergen, Purchase, NY.
c. American Bioresearch, Seymour, TN.
d. VMRD, Pullman, WA.

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