Analysis of genetic mutations in the 7a7b open reading frame of coronavirus of cheetahs (*Acinonyx jubatus*)

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Objective—To analyze the 7a7b genes of the feline coronavirus (FCoV) of cheetahs, which are believed to play a role in virulence of this virus.

Sample Population—Biologic samples collected during a 4-year period from 5 cheetahs at the same institution and at 1 time point from 4 cheetahs at different institutions.

Procedures—Samples were first screened for FCoV via a reverse transcription-PCR procedure involving primers that encompassed the 3′-untranslated region. Samples that yielded positive assay results were analyzed by use of primers that targeted the 7a7b open reading frames. The nucleotide sequences of the 7a7b amplification products were determined and analyzed.

Results—In most isolates, substantial deletional mutations in the 7a gene were detected that would result in aberrant or no expression of the 7a product because of altered reading frames. Although the 7b gene was also found to contain mutations, these were primarily point mutations resulting in minor amino acid changes. The coronavirus associated with 1 cheetah with feline infectious peritonitis had intact 7a and 7b genes.

Conclusions and Clinical Relevance—The data suggest that mutations arise readily in the 7a region and may remain stable in FCoV of cheetahs. In contrast, an intact 7b gene may be necessary for in vivo virus infection and replication. Persistent infection with FCoV in a cheetah population results in continued virus circulation and may lead to a quasispecies of virus variants. (*Am J Vet Res* 2006;67:627–632)

Feline coronavirus is a contagious and serious pathogen of Felidae. It is associated with mild to severe enteritis and is the etiologic agent of FIP, a fatal disease.[1,2] In domestic cat populations, FIP is a sporadic disease, although the causative virus is ubiquitous.[2] In contrast, outbreaks of FIP have been reported in several nondomestic species.[3–6] Enteritis associated with FCoV infection has resulted in mild to severe chronic diarrhea in several felid species and has been associated with vague signs of disease including weight loss, signs of depression, and inappetence.[4,7] Cheetahs (*Acinonyx jubatus*) are vulnerable to life-threatening disease following infection with FCoV.[4,8] Control of this pathogen is complicated by the occurrence of persistent infections, and carriers are an important source of the virus in cheetah populations.[7] In addition, detection of infection via currently available serologic assays does not identify all infected animals.[7] Management strategies for protection and preservation of cheetah populations require complete characterization of this virus.

Abbreviations

FCoV	Feline coronavirus
FIP	Feline infectious peritonitis
ORF	Open reading frame
RT-nPCR	Reverse transcription-nested PCR

The virus factors responsible for disease development are not known; however, virus factors are important in this regard because virus strains vary in virulence.[9] Virulent FCoV is theorized to arise from mutation of the infecting FCoV during replication in the intestinal tract of infected cats.[10,11] The 7b ORF is the 3′-most gene, and it is believed to have a role in virulence because deletions in this region lead to decreased virulence.[12] The 7a gene may also play a part in disease development; mutations in this region have been associated with an outbreak of FIP in a domestic cat population.[13] Despite these advances, the precise genetic basis for virulence of FCoV has not been characterized. The purpose of the study reported here was to analyze the 7a7b genes of the FCoV of cheetahs.

Materials and Methods

Sample collection—Samples were collected from 9 captive cheetahs located at 5 zoologic institutions in the United States. Of these 9, fecal samples from 3 healthy cheetahs and a sample of abdominal effusion from 1 cheetah with FIP were collected at 1 time point; these 4 animals resided at 4 different institutions. Also, fecal samples from 5 cheetahs housed together at a fifth institution were collected from May 1998 through July 2002. During this period, these 5 cheetahs had intermittent episodes of diarrhea, but no cases of FIP were identified in this population. All samples were stored at –70°C.

RNA extraction, RT-nPCR procedures, and sequence analysis—Total RNA was extracted from the specimens for reverse transcription by use of a total RNA isolation reagent (designed for use in liquid samples) according to the manu-

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facturer's directions.[a] Initial screening was done with primers that encompassed the 3′-untranslated region because this region is highly conserved among type I coronaviruses.[12] Samples for which results were positive were then amplified by use of primers that encompassed the 7a7b ORFs (ie, the 3′-most ORFs of the genome).[14] By use of the downstream external primer, reverse transcription was performed with Moloney murine leukemia virus reverse transcriptase according to the manufacturer's recommendations.[a] Nested PCR was done by use of *ExTaq* polymerase[b] as described previously.[14] Products were analyzed on a 1% agarose gel containing ethidium bromide.

Initially, amplified products from all 9 cheetahs were each cloned into pCR2.1 by use of a cloning system for single isolates.[c] A minimum of 3 clones from 2 RT-nPCR procedures were sequenced for each of the 9 isolates, including the 4 samples obtained from 4 cheetahs at separate institutions and 1 sample from each of the 5 cheetahs at 1 institution. The results from these latter samples led to collection of multiple samples during a 4-year period. The amplified products obtained from the multiple samples collected from 5 cheetahs over 4 years were purified by use of a PCR purification kit and directly sequenced.[d] Overall, the 7a7b regions in 40 isolates from this population were analyzed in this manner. All nucleotide sequencing was done by the Molecular Biology Resources Service of the University of Tennessee, Knoxville, Tenn, and analyzed by use of computer software.[e] Nucleic acid sequences corresponding to the 7a7b regions from all isolates were compared. The predicted amino acid sequences for this region were also compared to one another and to an FCoV isolate sequence from a domestic cat. The hydrophobicity and antigenicity profiles were generated according to the methods of Kyte and Doolittle[15] and Jameson and Wolf,[16] respectively.

Results

The 7a7b genetic ORFs of FCoV from 9 cheetahs were evaluated individually. Virus isolates from 5 cheetahs (cheetahs 1 to 5) at 1 institution were collected and analyzed during a 4-year period; additionally, isolates from the remaining 4 cheetahs (cheetahs 6 to 9,

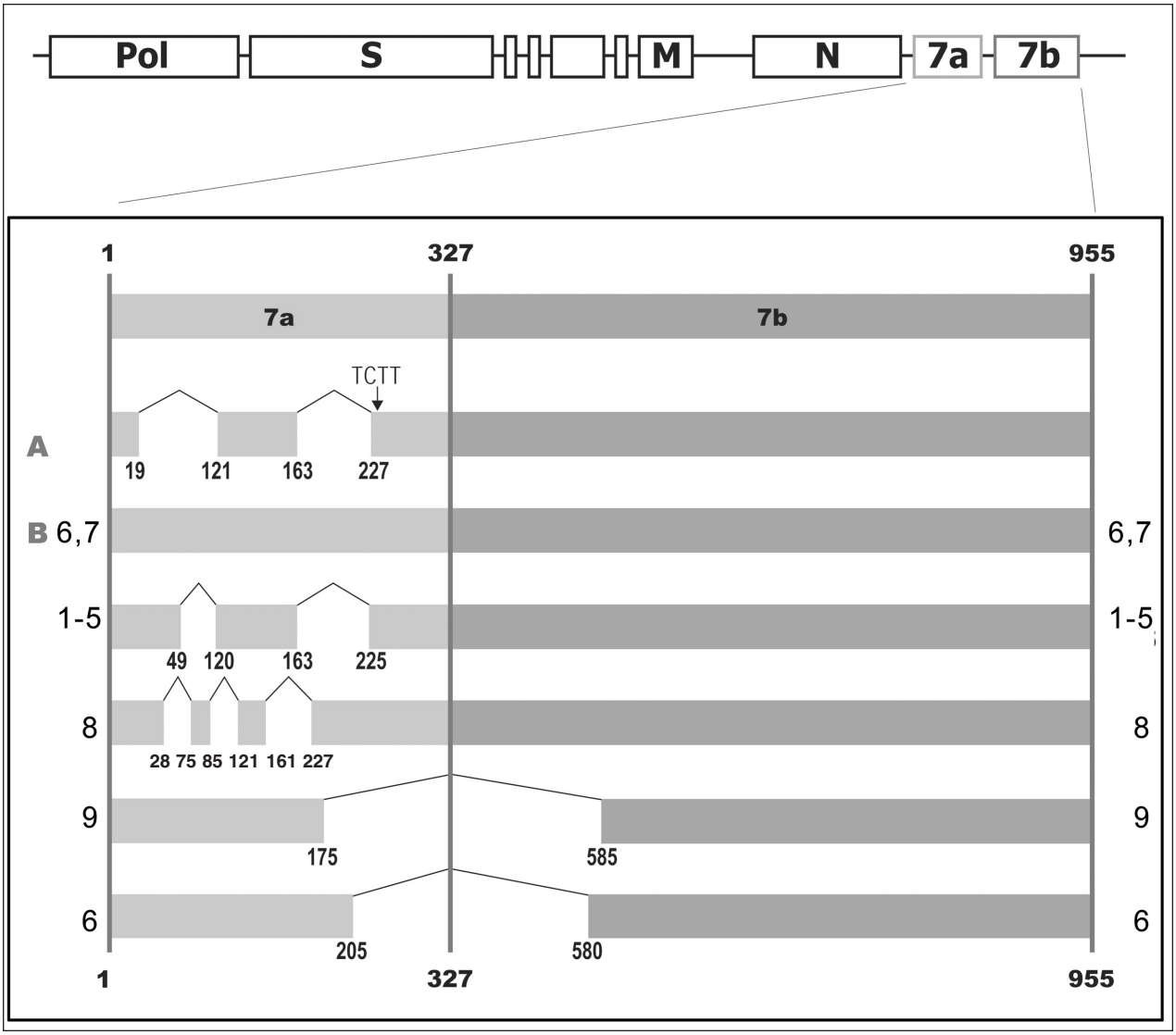

Figure 1—Representation of the 7a7b genes of FCoV. Schematic at the top of the image represents the location of the 7a7b region in the FCoV genome. A—7a7b genes from domestic cats.[13] Insertion of nucleotides in the domestic cat isolate is indicated by an arrow. B—7a7b genes of FCoV of 9 cheetahs (cheetahs 1 through 9) derived from fecal samples and a sample of abdominal effusion (cheetah 7). Numbers along rows represent nucleotide position in the 7a7b region. Pol = Polymerase gene. S = Spike gene. M = Membrane gene. n = Nucleocapsid gene.

which resided at 4 different institutions) were collected and analyzed at 1 time point (**Figure 1**). Each isolate was distinct in the 7a7b ORF region.

Cheetah 6 was infected with 2 variants, 1 with intact 7a7b genes and the other with a large deletion involving the 3′ third of the 7a gene and the 5′ third of

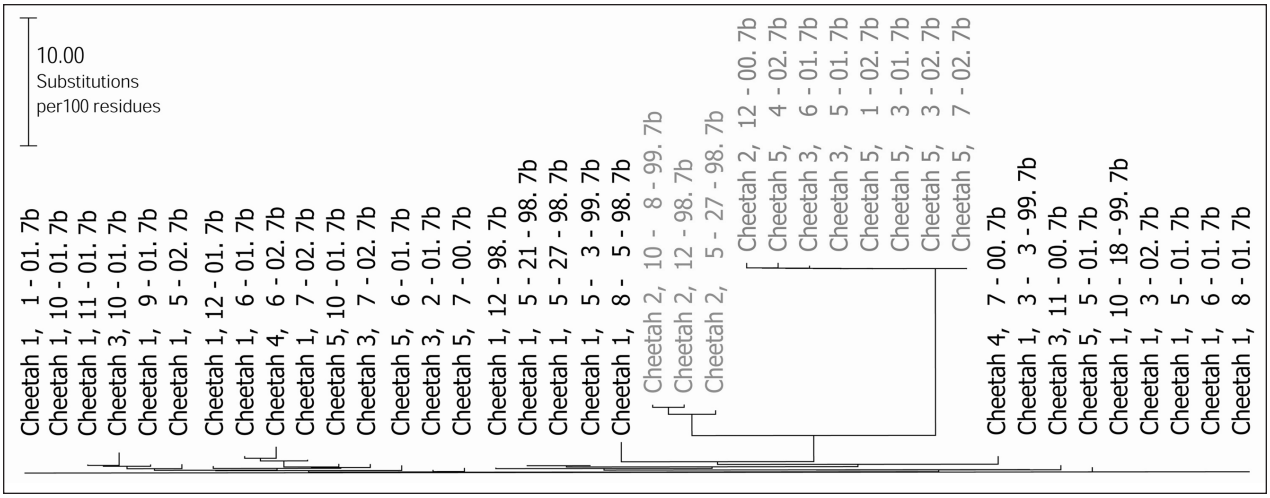

Figure 2—Phylogenetic tree of the 7b ORF in 40 FCoV isolates obtained from fecal samples collected during a 4-year period from 5 cheetahs (cheetahs 1 through 5) residing at the same zoologic institution. Cheetah number is followed by the date of sample collection.

Table 1—Variant detection among 40 isolates of feline coronavirus obtained from fecal samples collected during a 4-year period from 5 cheetahs (cheetahs 1 through 5) residing at the same zoologic institution. Variant A differed from variant B by the presence of 2 deletions. Variant A2 differed from variant A by the presence of point mutations equivalent to 4% of the nucleotides.

	Cheetah				
Date of sample collection	**1**	**2**	**3**	**4**	**5**
May 1998	A*	A2	N[z]	N	N
Jun 1998	U[i]	U	N	N	N
Jul 1998	U	U	U	N	U
Aug 1998	A	U	N	N	N
Dec 1998	A	A2	N	U	N
Mar 1999	A	ND	ND	ND	ND
May 1999	A	ND	ND	ND	ND
Jul 1999	U	ND	ND	ND	ND
Sep 1999	N	N	N	U	N
Oct 1999	A	ND	ND	ND	ND
Jul 2000	U	ND	N	A	A
Nov 2000	ND	ND	ND	ND	ND
Dec 2000	ND	B	ND	ND	ND
Jan 2001	A	ND	ND	ND	ND
Feb 2001	U	ND	A	ND	ND
Mar 2001	A	ND	ND	ND	ND
May 2001	A	ND	B	ND	A
Jun 2001	A	ND	B	U	A
Aug 2001	A	ND	U	N	U
Sep 2001	A	ND	ND	ND	ND
Oct 2001	A	ND	A	N	A
Nov 2001	A	ND	ND	ND	ND
Dec 2001	A	ND	N	ND	N
Jan 2002	U	ND	ND	ND	ND
Feb 2002	U	ND	U	ND	N
Mar 2002	A	ND	ND	ND	ND
Apr 2002	U	ND	N	N	B
May 2002	A	ND	ND	ND	ND
Jun 2002	A	ND	N	A	N
Jul 2002	A	ND	ND	ND	ND

*A, A2, and B indicate variant as determined by nucleotide sequencing.

N = Negative for virus by RT-nPCR assay. U = Positive for virus via use of UTR primers, but no amplification of 7a7b region. ND = Not done.

[z]Sample was FCoV-negative, as determined by use of an RT-nPCR assay. [i] Sample was FCoV-positive, as determined by use of untranslated region primers, but no amplification of 7a7b genes was achieved.

	1				50
Domestic Cat	M I V V L L V C V F	L A N G I K A T D V	Q H K P H E H P V L	T W E L L Q H F V G	H T L Y I T T N Q I
Cheetah 1 5/98 (A)	- - - I P - - - I -	M - - D V - - - I I	R K D - - - - - T -	- - D S - - - - - -	- - - - - - - - H - V
Cheetah 2 12/98 (A^2)	- L - I P - - - M S	- V - N V - - - I I	R S D - - - - - - -	- - - - - - - - A -	- - - H - A - H - -
Cheetah 2 12/00 (B)	- - - - - - - - - -	- - S - - - - - - -	- - D L - - - - - -	- - D - - - - - I -	- - - - - - - - H - -
	51				100
Domestic Cat	L A L P L G S R V E	C E S V E G F N C T	W P G F Q N P A H D	H V D F H F D L S N	P F Y S F V D N F Y
Cheetah 1 5/98	- - - - - - - - - -	- - - I - N - - - -	- - - - - K S - N -	- I - - Y - - - - S	- - - - - - - - S - -
Cheetah 2 12/98	- - - - - - - - - -	- - - I - N - - - -	- - - - - K S - N -	- I - - Y - - - - S	- - - - - - - - - -
Cheetah 2 12/00	- - - - - - - - - V	- - - - - - - - - -	- - - - - K - - Q -	- I - - Y - - - - D	- - - - - - - - - -
	101				150
Domestic Cat	I V G D G N Q R I N	L R L V G A V P K Q	K R L N V G C H T S	F A V D L P F G T Q	I Y H D R D F Q H P
Cheetah 1 5/98	- - - E - F - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - A - - -	- - Y - - - - - - -
Cheetah 2 12/98	- - - E - F - - - -	- - - I - - - - - -	- - - - - - - - - -	- - - - - - V - - -	- - - - - - - - - -
Cheetah 2 12/00	- - S E - D - - - -	- - - - - - - - - -	- - - - - - - Y - -	- - - - - - - - - -	- - - - - - - - Y -
	151				200
Domestic Cat	V S G R H L E C T H	R V Y F V K Y C P Y	N L H G Y C F N E K	L K V Y D L K Q L R	S K K V F D R L N Q
Cheetah 1 5/98	- G S - - - - - - -	- I - - - - - - - H	- - Y - - - - - - -	- - - - - - R - - -	- - - - - - K I - -
Cheetah 2 12/98	- - S - - - - - - -	- I - - - - - - - H	- - Y - - - - - - -	- - - - - - R - - -	- - - - - - E Q I - -
Cheetah 2 12/00	- - - - - - - - - -	- - - - L - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - L - K I - -

Figure 3—Multiple sequence alignment of the predicted amino acid sequence of the 7b protein from representative strains (variants A, A2, and B, respectively) of FCoV of cheetahs, compared with FCoV of domestic cats.[13] Amino acid matches to reference strain are indicated by a dash. Cheetah number is followed by the date of fecal sample collection. Numbers along rows represent predicted amino acid position within the 7a7b gene product.

the 7b gene. Cheetah 9 was infected with a single variant containing similar substantial deletions of the 7a and 7b genes. Such deletions are predicted to lead to abrogation of expression of the 7b product as a result of the start codon in affected isolates. The isolate from cheetah 7 had an intact 7a7b ORF. Interestingly, this cheetah died as a result of FIP. Cheetah 8 was infected with a variant that had 3 deletions in the 7a gene; these deletions were predicted to result in an alteration of the ORF leading to premature termination of the 7a product. All of these initial isolates, including the first samples from cheetahs 1 to 5, were characterized from cloned PCR products. On detection of virus shedding, samples were collected from cheetahs 1 to 5 during a 4-year period, and the resultant amplification products were sequenced directly.

One hundred four fecal samples from cheetahs 1 to 5 were collected over a 4-year period and analyzed via the RT-nPCR procedure. Samples were screened initially by use of primers that encompassed the 3′-untranslated region (a conserved genetic region), and 68 samples yielded positive results. During the 4-year period, results of sample analysis for 4 cheetahs were intermittently negative for FCoV, whereas for 1 cheetah, only 1 sample yielded negative results. Samples that were positive for FCoV were then used to amplify the 7a7b ORFs. In 28 FCoV-positive samples, failure to amplify the 7a7b region occurred. Forty isolates were successfully amplified by use of primers that encompassed the 7a7b ORFs, which were then analyzed and compared (**Table 1**).

Because of the proximity of the primers to the 7a start codon and 7b termination codon, the extreme 5′ and 3′ regions could not be analyzed. Two major virus variants were found to be circulating in this population: 1 with 2 major deletions in the 7a coding region (referred to as variant A) and 1 with an intact 7a gene (referred to as variant B). Within the A variants, 3 isolates of a subvariant (referred to as A2) obtained from cheetah 2 were identified; this subvariant differed in nucleotide identity from the other variant A isolates by approximately 4%. The difference between A2 and the other A variants comprised 1 span of 5 nucleotides and 19 point mutations. The segregation of isolates was also evident via examination of the 7b region only. Within the variant A group, homology of ≥ 97% was detected and variant A2 was 95% to 96% homologous with variant A; in the 7b region, variant B was 86% homologous with variant A. Within each group of variants or subvariants, the extent of nucleotide homology for the 7a7b region was ≥ 98%, with variation due to point mutations. Thus, 2 distinct clades were identified (variants A and B), with an additional subvariant (A2) detected in 1 cheetah (**Figure 2**).

All 3 variants appeared to be circulating simultaneously because each was detected at multiple time points throughout the study period (Table 1). Variant B was not detected until December 2000 in 1 cheetah followed by its appearance in 2 additional cheetahs in March and May 2001. Only 1 variant, the deletion mutant A, was identified from 2 cheetahs (cheetahs 1 and 4), whereas the other 3 cheetahs were infected with and shed > 1 variant during the study period.

In 24 isolates obtained from the 1 cheetah from which samples consistently yielded positive results during the 4-year evaluation period, only 2 nucleotide changes in the 7b region were detected over that interval; thus, 2 of 24 isolates differed from the remaining isolates by 1 nucleotide each.

Computer-generated translation of the 7a gene was done for the deletion mutants of cheetahs 1 to 5, but because the 5′ end of the gene was not sequenced and corresponded to the internal upstream primer, the presence of the start codon could not be assessed. The predicted amino acid sequence of the available nucleotides indicated the presence of a premature termination signal (data not shown). This resulted from changes in the reading frame as a result of the deletional mutations. The predicted amino acid sequences of the 7b gene from the 3 representative strains were compared with the sequence of the same region of domestic cats (**Figure 3**). Multiple point mutations leading to amino acid differences were identified. Hydrophobicity and antigenicity profiles of the predicted amino acid sequence for the 7b protein of the 3 representative cheetah coronaviruses (variants A, A2, and B) were generated according to the methods of Kyte and Doolittle[15] and Jameson and Wolf,[16] respectively (data not shown). These point mutations did not appear to result in alteration of the protein hydrophobicity. Minor variations in antigenicity of the variants were detected.

Discussion

In the present investigation, deletional mutations were identified in the 7a and 7b genes of the FCoV of cheetahs. Notable deletions in the 7a region were found in most of the isolates. Because the internal upstream sequencing primer coincided with the first 20 nucleotides of the 7a gene, it could not be determined what, if any, changes had occurred in this region. However, the deletions that were identified involved substantial genetic regions of the 7a gene resulting at best in the expression of a truncated aberrant protein because of the change in reading frame and resultant premature termination signal. The FCoV from 2 cheetahs had a major deletion involving the 7a7b ORFs that resulted in a truncated 7a gene product and complete abrogation of expression of the 7b product because of loss of the start codon. These variants were from 1 time point and were identified via sequencing of the cloned products rather than direct sequencing of the RT-nPCR products. Therefore, it is not known whether these variants were present in considerable numbers in the population of viruses shed by these cheetahs or represented a minor variant that may be defective as a result of the major deletion in this region. Interestingly, the only cheetah with FIP had intact 7a and 7b genes. However, viruses from 4 healthy cheetahs also had intact 7a and 7b genes. Thus, this region may be necessary but not sufficient for the development of disease. Analysis of additional isolates from cheetahs with FIP would be necessary to establish an association between the intact 7b ORF and disease development. In addition, other genomic regions should be analyzed to determine their possible role in virulence.

Several cheetahs were found to be infected with > 1 FCoV variant. This occurrence of multiple virus variants in an individual cheetah was similar to reported findings in individuals within a domestic cat population.[13] This could result from superinfection with a different strain or mutation of the infecting strain that gives rise to a different variant.

One hundred four fecal samples that were collected from 1 population of cheetahs over a 4-year period were used for virus detection in our study; of these, 68 samples yielded positive results for FCoV. In this population of 5 cheetahs, 4 were shedding virus intermittently; 1 cheetah was shedding virus consistently, and during the entire study period, only 1 FCoV-negative sample was collected from this animal. We speculated that this cheetah was persistently infected. The FCoV-positive samples were used for amplification of the 7a7b ORFs, but this region could not be amplified from 28 of those samples. Given that deletional mutations were identified near the internal upstream primer-binding site, this site may have been lost in some viruses. Alternatively, the viral RNA in those 28 samples may have been degraded in the time between initial screening and 7a7b amplification.

The 7a7b region was successfully amplified from 40 isolates. Two major virus variants were identified—1 with 2 major deletions in the 7a gene (variant A) and another with an intact 7a gene (variant B). Within the variant A deletional mutant group, a subvariant (variant A2) with minor genetic differences (approx 4% nucleotide variation) was detected. Within the A and A2 groups of isolates, minor nucleotide differences (≤ 2%) were identified, indicating the presence of a quasispecies of virus in the infected cheetahs. Each of the major variants (A, A2, and B) was detected at multiple time points over the 4-year period, which suggested that all were circulating simultaneously, though variant B was not detected until December 2000. In 2 cheetahs (including the persistently infected cheetah), only 1 variant, the deletional mutant A, was detected. Because all the animals were housed together, it is likely that these 2 cheetahs were also infected with the intact variant, but they may have cleared the virus, or the mutant variant may have been present in higher amounts than the intact variant. Sequencing of amplification products provides the consensus sequence of the most prevalent virus in the sample; small amounts of other variants may be undetected.

In the present study, the mutations detected in the 7a gene were similar among most isolates obtained from the cheetahs and were similar to virus from a domestic cat population.[13] We speculated that the secondary structure in the RNA corresponding to this region may cause the viral RNA polymerase to skip over these regions during transcription. These mutations were highly stable (because they were unchanged throughout the study period), as was the remainder of the 7a coding region among all isolates. The resultant deletional mutations in the 7a gene of variant A were predicted to result at least in an aberrant protein and at most in complete abrogation of 7a expression. In contrast, the 7b gene was intact (with minor sequence variation) among all isolates in the cheetah population. These 7b gene variations segregated in the same pattern as the 7a region, with the 7b region of the 7a deletional mutants being distinct from the 7b region of isolates with an intact 7a gene. Within each group of variants, multiple point mutations were identified, indi-

cating that the virus continued to change with continued replication; the result was a quasispecies of different isolates, most of which had considerable homology with each other. On comparison of predicted amino acid sequences from representative isolates, minor differences were revealed. These resulted in alteration in antigenicity that is believed to be unimportant.

The lack of substantial mutations in the 7b gene of FCoV in this population of cheetahs would seem to indicate that it is essential for virus infection or replication. Notably, among 24 isolates from 1 cheetah collected during the 4-year study period, only 2 point mutations were detected, suggesting remarkable conservation for an RNA virus. This cheetah may have been resistant to superinfection with other variants. The occurrence and apparent stability of viruses with major deletions in the 7a gene would imply that the 7a gene product may be a nonessential protein for replication. The role of this product in disease development remains unclear. It is also not known whether the 7a deletional mutant was derived from the 7a intact variant, but the lack of notable sequence variation in the 7b region among all isolates would imply a shared ancestry. However, the intact variant was not identified in fecal samples from cheetahs in the present study until December 2000, indicating it could have entered the population at that time. This corresponded to the transfer of this cheetah group to a new locale; thus, they may have been exposed to a new virus at that time. The virus may have been present in the enclosure (a large natural area not amenable to disinfection), having been introduced by a previous inhabitant.

The cheetahs in the population included in the present study appeared to be persistently infected; alternatively, infection, viral clearance, and reinfection may have been occurring among the animals. However, fecal samples from 1 cheetah yielded positive results consistently, which implies persistent infection in that animal. During the study period, cheetahs in this population had intermittent episodes of diarrhea, in which FCoV may have played a role.

Our data indicated that deletional mutations occurred with great frequency in the 7a gene of the FCoV of cheetahs, whereas the 7b gene was more genetically conserved. The continued replication of variants with deletions in the 7a gene indicates it is not essential for virus infection or replication. Although the 7b region varies among isolates, these mutations do not appear to result in important alterations within the resultant protein, suggesting that this protein may be essential for virus infection or replication. Infected cheetahs were found to harbor a quasispecies of viruses. Some cheetahs remained persistently infected and could represent an important source of genetically mutated virus. In populations containing persistently infected cheetahs, virus may circulate among animals for extensive periods of time. Continuous replication of viruses may lead to the emergence of mutants that may vary in virulence. Regular evaluation of cheetahs and removal of chronically infected animals may thus be an important management tool with which to minimize the incidence of FCoV-induced disease. Future studies characterizing additional isolates associated with FIP are warranted to determine the role of genetic mutations in disease development among cheetahs.

a. Gibco BRL, Baltimore, Md.
b. Intergen, Purchase, NY.
c. Invitrogen, Carlsbad, Calif.
d. MinElute PCR purification kit, Qiagen Inc, Valencia, Calif.
e. GCG sequence analysis software, University of Wisconsin, Madison, Wis.

References

1. Hoskins JD, Loar AS. Coronavirus infection in cats. *Vet Clin North Am: Sm Anim Pract.* 1993;1–16.
2. Pedersen NC. An overview of feline enteric coronavirus and infectious peritonitis virus infections. *Feline Pract* 1995;23:7–20.
3. Evermann JF. Feline coronavirus infection of cheetahs. *Feline Pract* 1986;16:21–30.
4. Heeney JL, Evermann JF, McKeirnan AJ, et al. Prevalence and implications of feline coronavirus infections of captive and free-ranging cheetahs (*Acinonyx jubatus*). *J Virol* 1990; 64:1964–1972.
5. Watt NJ, MacIntyre NJ, McOrist S. An extended outbreak of infectious peritonitis in a closed colony of European wildcats (*Felis silvestris*). *J Comp Pathol* 1993;108:73–79.
6. Juan-Salles C, Domingo M, Herraez P, et al. An outbreak of feline infectious peritonitis in captive servals (*Felis serval*): clinical, pathological, and immunohistochemical findings. *Proc Am Assoc Zoo Vet* 1997;224–226.
7. Kennedy MA, Citino S, Dolorico T, et al. Serology and genetic detection of feline coronavirus of cheetahs (*Acinonyx jubatus*) in the USA. *J Zoo Wildl Med* 2001;32:25–29.
8. Evermann JF, Heeney JL, McKeirnan AJ, et al. Comparative features of a coronavirus isolated from a cheetah with feline infectious peritonitis. *Virus Res* 1989;13:15–28.
9. Fiscus SA, Rivoire BL, Teramoto JA. Humoral immune response of cats to virulent and avirulent feline infectious peritonitis virus isolates. *Adv Exp Med Biol* 1987;218:559–568.
10. Poland AM, Vennema H, Foley J, et al. Two related strains of feline infectious peritonitis virus isolated from immunocompromised cats infected with a feline enteric coronavirus. *J Clin Microbiol* 1996;34:3180–3184.
11. Vennema H, Poland A, Foley J, et al. Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. *Virology* 1998;243:150–157.
12. Herrewegh AAPM, Vennema H, Horzinek MC, et al. The molecular genetics of feline coronavirus: comparative sequence analysis of the ORF7a/7b transcription unit of different biotypes. *Virology* 1995;212:622–631.
13. Kennedy MA, Boedeker N, Gibbs P, et al. Deletions in the 7a ORF associated with an epidemic of feline infectious peritonitis. *Vet Microbiol* 2001;81:227–234.
14. Kennedy MA, Brenneman K, Millsaps RK, et al. Correlation of genomic detection of feline coronavirus with various diagnostic assays for feline infectious peritonitis. *J Vet Diagn Invest* 1998;10:93–97.
15. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol* 1982;157:105–132.
16. Jameson BA, Wolf H. The antigenic index: a novel algorithm for predicting antigenic determinants. *Comput Appl Biosci* 1988;4:181–186.