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Abstract: The extent and progression of exposure to feline infectious peritonitis (FIP) virus in the cheetah, *Acinonyx jubatus*, was monitored by a world-wide serological survey with indirect fluorescent antibody titers to coronarvirus. The indirect fluorescent antibody assay was validated by Western blots, which showed that all indirect fluorescent antibody-positive cheetah sera detected both domestic cat and cheetah coronarvirus structural proteins. There was a poor correlation between indirect fluorescent antibody results and the presence of coronaviruslike particles in cheetah faeces, suggesting that electron microscopic detection of shed particles may not be an easily interpreted diagnostic parameter for FIP disease. Low, but verifiable (by Western blots [immunoblots]) antibody titers against coronavirus were detected in eight free-ranging cheetahs from east Africa as well from captive cheetahs throughout the world. Of 20 North American cheetah facilities screened, 9 had cheetahs with measurable antibodies to feline coronavirus. Five facilities showed patterns of an ongoing epizootic. Retrospective FIP virus titers of an FIP outbreak in a cheetah-breeding facility in Oregon were monitored over a 5-year period and are interpreted here in term of clinical disease progression. During that outbreak the morbidity was over 90% and the mortality was 60%, far greater than any previously reported epizootic of FIP in any cat species. Age of infection was a significant risk factor in this epizootic, with infants (less than 3 months old) displaying significantly higher risk for mortality than subadults or adults. Based upon these observations, empirical generalizations are drawn which address epidemiologic concerns for cheetahs in the context of this lethal infectious agent.

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Prevalence and Implications of Feline Coronavirus Infections of Captive and Free-Ranging Cheetahs (*Acinonyx jubatus*)

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The extent and progression of exposure to feline infectious peritonitis (FIP) virus in the cheetah, *Acinonyx jubatus*, was monitored by a world-wide serological survey with indirect fluorescent antibody titers to coronavirus. The indirect fluorescent antibody assay was validated by Western blots, which showed that all indirect fluorescent antibody-positive cheetah sera detected both domestic cat and cheetah coronavirus structural proteins. There was a poor correlation between indirect fluorescent antibody results and the presence of coronaviruslike particles in cheetah feces, suggesting that electron microscopic detection of shed particles may not be an easily interpreted diagnostic parameter for FIP disease. Low, but verifiable (by Western blots [immunoblots]) antibody titers against coronavirus were detected in eight free-ranging cheetahs from east Africa as well as from captive cheetahs throughout the world. Of 20 North American cheetah facilities screened, 9 had cheetahs with measurable antibodies to feline coronavirus. Five facilities showed patterns of an ongoing epizootic. Retrospective FIP virus titers of an FIP outbreak in a cheetah-breeding facility in Oregon were monitored over a 5-year period and are interpreted here in terms of clinical disease progression. During that outbreak the morbidity was over 90% and the mortality was 60%, far greater than any previously reported epizootic of FIP in any cat species. Age of infection was a significant risk factor in this epizootic, with infants (less than 3 months old) displaying significantly higher risk for mortality than subadults or adults. Based upon these observations, empirical generalizations are drawn which address epidemiologic concerns for cheetahs in the context of this lethal infectious agent.

Feline infectious peritonitis (FIP) is a fatal immune-mediated disease of domestic cats that is a consequence of infection by an immunogenic coronavirus, FIP virus (FIPV), with a positive-stranded RNA genome. The epizootiology and etiology of FIP are not well understood, despite considerable study since the original description in 1963 (3, 10, 15, 37, 38). At least three clinical forms of disease are recognized: (i) effusive or wet FIP, which is characterized by fibrinous peritonitis or pleuritis and which is always fatal; (ii) the noneffusive or dry form of FIP, which does not have the fluid but which does have the fibrinous peritoneal deposition and is also fatal; and (iii) a subclinical enteritis, which is mild in terms of recognizable symptoms (27; J. E. Barlough and C. A. Stoddart, *in* C. E. Greene, ed., *Infectious Diseases of the Dog and Cat*, in press). In domestic cats the fatal form is rare (ca. 1% of an infected colony will die), and the virus seldom affects more than 10% of domestic cats in a group even under the most severe conditions for disease (27). Several FIPV isolates have been described, and they may vary from extreme virulence and pathology to subclinical outcomes (5, 21, 27, 28). The non-FIP forms of feline coronavirus have been designated feline enteric coronavirus (FECV) to distinguish them from immunologically related but pathological FIPV isolates (20, 21, 27, 28, 36; Barlough and Stoddart, in press).

Before 1982, serological evidence established that cheetahs (*Acinonyx jubatus*) were susceptible to infection by feline coronaviruses (FIPV, FECV, and other related strains), but no cases of clinical FIP were reported (1, 16). Beginning in 1982, a devastating epizootic occurred in a cheetah-breeding colony located at Wildlife Safari in Winston, Oreg. (2, 6, 9, 31). What initially appeared as an acute anorexia, jaundice, and enteritis in an adult cheetah resulted in death from FIP and rapid viral transmission to other resident cheetahs. Within 6 months, every cheetah in the facility developed immunofluorescent antibodies to feline coronavirus, ostensibly because of exposure to the fatal FIPV that had afflicted the original cheetah (2, 9, 23). Over the next 12 months, clinical signs (intermittent and chronic diarrhea, chronic gingivitis, hepatic and renal disease, weight loss, and depression) and morbidity were apparent in over 90% of the cheetahs. Despite aggressive clinical therapy, a total of 27 cheetahs died between 1983 and 1987 from one or more of the following coronavirus-associated diseases: fibrinous peritonitis, renal and hepatic disease, enteritis and malabsorption syndrome, hemorrhagic gastroenteritis, and FIP-associated kitten mortality complex. In the same period, 18 cheetahs were exposed to the FIPV and

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survived, thereby permitting an overall mortality estimate of 60%. Since the Oregon epizootic, and perhaps in some part because of it, the understanding of the etiology of FIP in cheetahs has become an important priority in discussions of captive breeding and wildlife management strategies for the species (8, 19). The inability to develop an effective vaccine or treatment for FIP has made this virus a serious concern that must be considered in programs designed to stabilize and protect cheetah populations.

We present here an update on the extent and progress of the Oregon FIP epizootic from 1982 through 1987 which serves as an important model for interpreting immunological data in other populations. In addition, we present a serologic survey of the prevalence of feline coronavirus infection in captive cheetahs from zoological facilities in North America, Europe, East Africa, and South Africa plus a population survey from free-ranging cheetahs of the Serengeti ecosystem. The various feline coronavirus detection techniques, including immunofluorescence, Western blots, and electron microscopy, were compared, and the epizootical results were interpreted in terms of management recommendations for this severely threatened species.

MATERIALS AND METHODS

Serological assays. Serum samples were collected from 45 cheetahs at Wildlife Safari in Winston, Oreg., on a yearly basis since 1982. In addition, serum samples were submitted from 132 cheetahs held at 20 zoologic facilities throughout the United States and Canada (18). Serum was also collected from 101 cheetahs in Africa and Europe. There were two sites in eastern Africa, one site in southern Africa, and one site in Great Britain. Antibodies against the feline coronaviruses were measured by an indirect fluorescent antibody (IFA) assay with canine coronavirus (I-71 strain) as the antigen substrate (2, 6, 9). Previous studies assessing feline coronavirus antibody titers in serum from domestic cats and cheetahs have shown that there is considerable antigenic similarity when the substrate is prepared with either canine coronavirus, transmissible gastroenteritis virus of swine, or FIPV (16, 30). The substrate was prepared in Crandell feline kidney (CrFK) cells set on microscope slides with an 8-well Bellco template (Bellco, Vineland, N.J.). The slides were fixed in chilled acetone for 10 min, air dried, and stored at −20°C until serologic tests were run. Cheetahs with IFA titers in serum of 1:25 and higher were considered seropositive to feline coronavirus (6).

EM. Fecal samples from cheetahs located in facilities in North America were processed and observed by electron microscopy (EM) as previously described (33). Fecal samples were refrigerated during shipment to the laboratory and stored at 4°C before analysis to avoid freezing, which can alter virus morphology.

Western blots. The Western blot (immunoblot) analyses were performed by the method of Towbin et al. (35). Briefly, virus samples were treated with 2× phosphate-buffered saline and electrophoresed for 60 min at 200 V (constant voltage) in 12% sodium dodecyl sulfate–polyacrylamide gels in a Bio-Rad mini-gel apparatus (Bio-Rad Laboratories, Richmond, Calif.) equilibrated for 30 min in 25 mM Tris–192 mM glycine–20% methanol (transfer buffer). Proteins were transferred by electric charge to nitrocellulose paper (Scheller & Schuell, Inc., Keene, N.H.) in a minitransfer apparatus (Bio-Rad) in transfer buffer at 30 V (constant voltage) overnight. Blots were disassembled and briefly washed in TBS (20 mM Tris, 500 mM NaCl [pH 7.4]) for 10 min, blocked in 3% gelatin–TBS, and reacted with cheetah or domestic cat sera to detect the presence of coronavirus antibodies. After two 10-min washes, blots were immersed in 1:100 dilution of anti-cat whole immunoglobulin G (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) in 1% gelatin–TBS for 60 min, washed twice, and reacted with BCIP/NBT alkaline phosphatase substrate system (Kirkegaard and Perry Laboratories) for 15 min. Blots were scored 0 to 3 based on the intensity of bands identified at 205 kilodaltons (nucleocapsid) and 24 kilodaltons (membrane proteins).

Viruses. Comparison of antigenic profiles identified by seropositive cheetahs with seropositive domestic cats was conducted by using four pathologic strains of domestic feline coronavirus. The strains included FECV WSU 79-1683, FIPV WSU 79-1146, FIPV NOR-15, and FIPV UCD-1 (5, 21, 27, 29). The cheetah coronavirus isolate AJUCV-1 (previously designated WSU 83-4497) was maintained in CrFK cells as a persistent nonlytic infection (6, 7). Supernatants from cells infected with the four feline coronavirus strains and from cells persistently infected with WSU 83-4497 were harvested and processed by differential centrifugation. Briefly, FC-009 cells (courtesy of N. C. Pedersen) were grown in Dulbecco minimal essential medium, and 10% fetal bovine serum was used to propagate the four feline coronavirus strains, which were inoculated at a multiplicity of infection of approximately 0.1. The supernatant was harvested from flasks when 80 to 90% cytopathic effect was obtained and stored at −20°C. After clarification at 800 × *g* at 4°C for 20 min, the supernatant was carefully removed, and the virus was pelleted at 16,500 rpm 4°C for 45 min. Samples of 100×-concentrated virus were mixed with 2× sample buffer and run on 12% sodium dodecyl sulfate–polyacrylamide gels and stained with Coomassie blue to quantify virus antigens.

RESULTS

An epizootic of FIP in captive cheetahs. The Wildlife Safari in Winston, Oreg., began what was to become a highly successful cheetah-breeding program in 1973. In May 1982, two cheetahs, studbook (SB) numbers 79 and 80 (18), were imported into the park. Within a few weeks SB79 developed severe jaundice, depression, fever, and diarrhea and died in late June of FIP (31). A retrospective survey of cheetah serum collected from the facility's cheetahs before May 1982 revealed that none (of 25 tested) had circulating antibody titers against coronavirus, based upon an IFA assay. Within 6 to 8 months, sera from every cheetah in the facility were positive for antibodies to coronavirus, a result consistent with dynamic highly contagious infection with FIPV.

A summary of the antibody titer progression of 45 cheetahs that became infected during the period from 1982 through 1988 is presented in Fig. 1. Figure 1a presents the titers of cheetahs who succumbed to FIP-related disease (n = 27), and Fig. 1b presents the titers of cheetahs that developed antibodies to coronavirus but did not die of frank FIP as of June 1988 (n = 18). Preliminary reports of earlier studies have been presented elsewhere (2, 9).

There are several important observations which emerge from examination of Fig. 1. First, before May 1982 each tested animal was seronegative (FIPV antibody titers, <25), and after 6 months all cheetahs became seropositive. Second, with time antibody titers in all cheetahs increased to

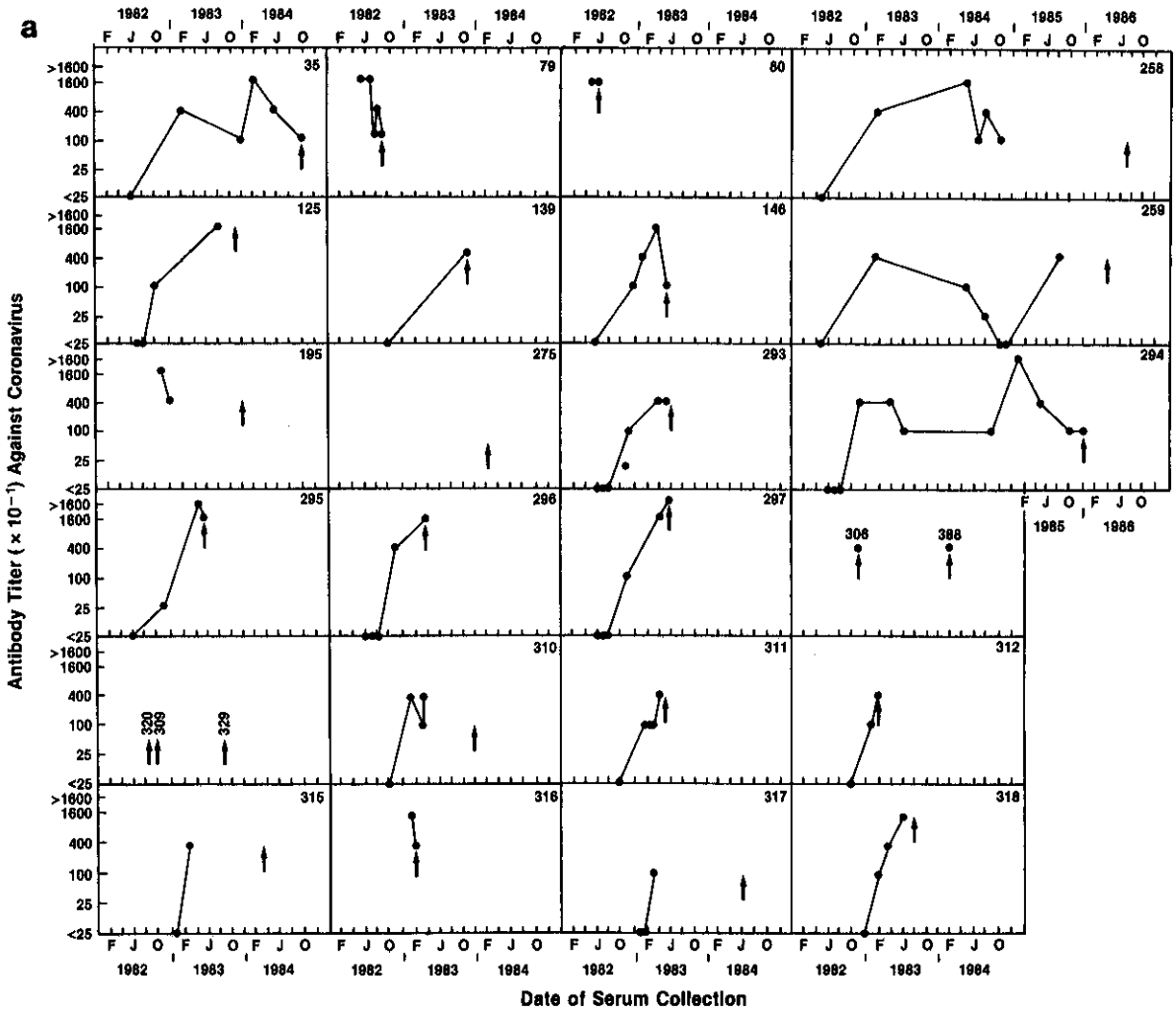

FIG. 1. Time course of FIP IFA titers in serum from cheetahs at Wildlife Safari, Winston, Oreg. Studbook numbers are from the North American cheetah studbook (18). Arrows indicate dates of death. SB79 and SB80 were the two animals that arrived at the park with FIP titers. (a) Animals that have died of FIP-related disease based upon necropsy diagnosis. (b) Animals that have been exposed acutely but recovered and were without symptoms at the last sampling.

between 1:400 and 1:1,600. Third, there was often a modest decrease in FIPV antibody titer in dying animals, presumably as a consequence of immune suppression during later disease stages. Fourth, there was no obvious difference in the seroprevalence patterns in animals that succumbed to FIPV and those that survived. Fifth, in surviving animals, antibody titers tended to persist for several years, suggesting a chronic infection with the FIPV which continually stimulated the immune system of infected cheetahs. There were certain exceptions to this pattern (e.g., SB319 and SB383), but the more common scenario was persistence of appreciable antibody titers against coronavirus for a period of 4 to 6 years.

An important risk factor that would influence mortality due to FIPV appears to be age at exposure (see reference 18 for dates of birth). Of the 45 cheetahs studied, 20 were exposed as infants (less than 5 months old); of these, only 3 (15%) survived. The survival rate of 25 older cheetahs was 60%. The overall mortality related to FIPV exposure was 60%. The relative survival of infants exposed to FIPV was significantly different from that for all cheetahs ($\chi^2 = 9.37$; $P < 0.01$).

Among the cheetahs which succumbed to FIP, the median time from seroconversion to death was 7 to 12 months (Fig. 2). Three animals, SB258, SB259, and SB294, each survived over 38 months before dying of FIP, but their FIPV antibody titer patterns are perhaps illuminating. All three cheetahs showed declining antibody titers in 1983 through 1984 (the titer of SB259 dropped to ≤25 for two consecutive samples in 1984), and thereafter the two tested animals developed elevated titers in 1985 until their deaths in 1986 (Fig. 1a). Since the three animals were housed in the same pens, their parallel pattern may indicate an acute secondary infection or possibly a common environmental change that caused activation of latent FIPV. The other surviving cheetahs have lived for up to 5 years (Fig. 2b) without apparent symptoms.

Seroprevalence in captive and free-ranging cheetah populations. Coronavirus antibody titers from 101 cheetah serum samples collected from four locations outside of the United States are presented in Table 1. With the exceptions of sera collected in the Serengeti from 25 free-ranging cheetahs (25), the sera were from captive animals, although several of these cheetahs in Africa were born in the wild. Low antibody titers were detected in a few animals from each locale, but only

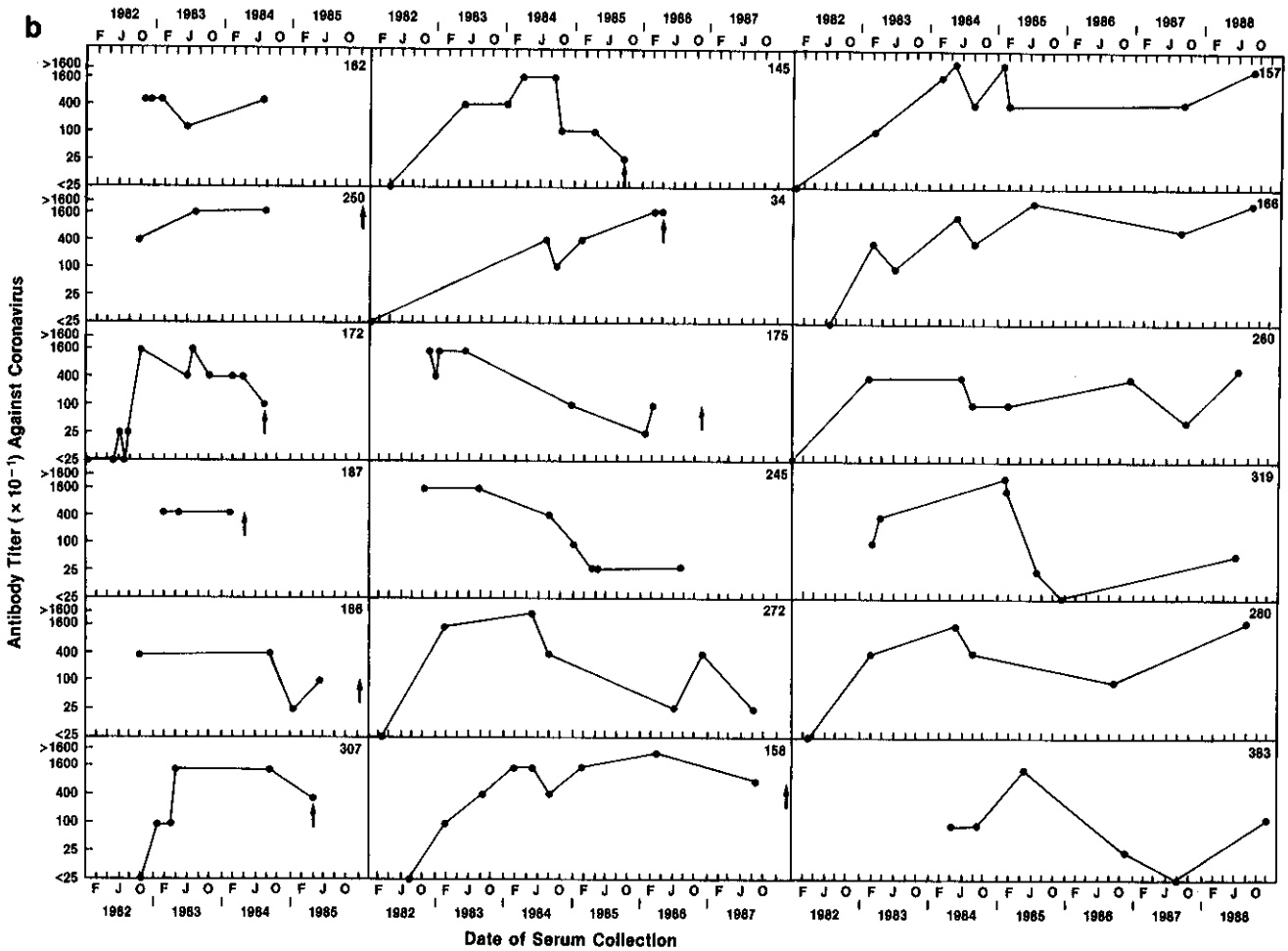

FIG. 1—*Continued.*

two cheetahs (one from the Serengeti and one captive animal in Kenya) had titers of 1:100 or greater. Low IFA titers (1:25) were verified by Western blots (see below). With the exception of these infrequent low titers, the overall titer pattern in each of these locations resembles the Oregon situation before 1982 (Table 1).

In the United States, the Oregon epizootic influenced the cheetah species survival plan, organized by the American Association of Zoological Parks and Aquariums, to recommend periodic monitoring of captive cheetahs for coronavirus titers. The distribution of feline coronavirus titers in serum samples from 132 cheetahs held in 20 North American facilities (zoos and wildlife reserves) is presented in Table 2.

Eight of the facilities provided samples of their colonies in both 1986 and 1987 (1 through 8 in Table 2). Among these facilities, there were two patterns that emerged: (i) those in which all cheetahs were clearly seronegative over time (facilities 1 through 4); and (ii) those in which cheetahs were clearly seropositive in 1986 (facilities 5 through 8), with a progression from low titers in a few animals to higher titers in more animals. Like the experience at Wildlife Safari, the FIPV appears to spread to other cheetahs in these facilities and to proceed with increasing coronavirus titers. Interestingly, animals in these four facilities did not exhibit clinical symptoms of FIP, although FIP pathology has been observed in two other North American facilities not monitored serologically in our studies (19) as well as in two sites in Europe and three facilities in Japan (Marker-Kraus et al., manuscript in preparation). The remaining surveyed institutions only produced one-time samples, so the results were less easily interpreted. However, cheetahs in facilities 9 through 15 produced low or no titers, similar to those from DeWildt and Whipsnade, whereas facilities 16 through 20 maintained cheetahs with higher titers more reminiscent of those at Wildlife Safari in the years after the FIP outbreak.

IFA titers do not correlate with EM screens for coronaviruslike particles. Seventy-four fecal samples were submitted from captive cheetah populations at 13 facilities in North America for EM analysis. The overall prevalence of cheetahs with evidence of fecal coronaviruslike particles was 31% (21 of 68) (Table 2). Of the 21 cheetahs shedding coronaviruslike particles in their feces, only 8 (38%) had demonstrable serum IFA titers to coronavirus. The high frequency of discordance between feline coronavirus antibodies and the presence of fecal particles is difficult to explain but could be the result of the occurrence of coronaviruslike particles in the feces which are immunologically distinct from the pathological FIPV.

Virus isolation was attempted by using CrFK cells on all fecal samples in which particles were observed. None of these attempts, however, was successful. Previous coronaviral isolations from both domestic cats and cheetahs have revealed a strong dependence upon tissue of origin, host cell

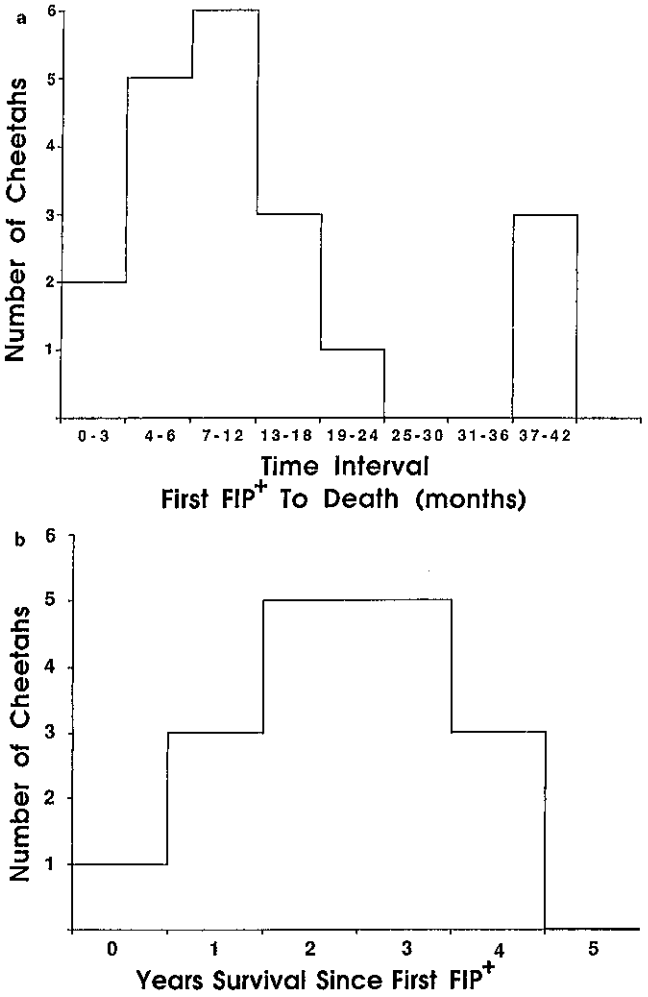

FIG. 2. (a) Distribution of the time intervals between infection with FIPV and death by FIP-related disease in cheetahs at Wildlife Safari, Winston, Oreg. (b) Time of survival since FIPV infection in surviving cheetahs.

system, and the presence of exogenous protease to enhance viral replication (5–7, 20).

Western blot analyses. Serum samples (diluted 1:5, 1:10, 1:50, and 1:100) from 93 African cheetahs (Table 3) plus from 15 cheetahs from Wildlife Safari were screened by using Western blots against four feline coronavirus strains (Fig. 3). All IFA-positive animals, except one with a titer of 1:100, detected coronavirus proteins of each FIPV isolate on Western blots. The blot profile varied for each isolate, most appreciably in the membrane (or matrix) protein region, where a common 29-kilodalton band was detected in strains FIPV WSU 79-1146, FIPV Nor-15, and FECV WSU 79-1683 but was faint or absent in the FIPV UCD-1 preparation. The intensity of staining of each band was a function of antibody titer, but there were also some noticeable differences and similarities between individuals on different continents. For example, the isolate from an East African cheetah (AJU 220) showed a major 29-kilodalton band like that of the Nor-15 strain and had the same Western blot profile as the isolate from one captive North American cheetah (AJU 87) but differed from the isolate from another East African cheetah (AJU 219) which failed to show the 29-kilodalton protein (Fig. 3).

TABLE 1. Worldwide prevalence of cheetah coronavirus exposure

Location	Date serum collected	No. of cheetahs with coronavirus titers[a] of:				
		≤25	25	100–125	400–625	≥1,600
DeWildt, South Africa[b]	1982	49	3	0	0	0
Serengeti, East Africa[c]	1985	17	7	1	0	0
Whipsnade, England[d]	1985	11	1	0	0	0
Kenya captive, East Africa	1985	8	3	1	0	0
Wildlife Safari, Oreg.	Before June 1982	25	0	0	0	0
	June 1983	0	0	2	18	15
	June 1985	0	1	5	7	3

[a] IFA titers are expressed as the reciprocal of the highest dilution of serum resulting in positive immunofluorescence.
[b] DeWildt Cheetah Research Center, Transvaal, Republic of South Africa.
[c] Free-ranging animals collected in the Serengeti National Park and the Ngorongora Conservation Reserve in Tanzania (25).
[d] Whipsnade Park, a preserve operated by the Zoological Society of London.

The numbers of African cheetah serum samples that detected antigens of the four isolates of domestic cat coronavirus are presented in Table 3. This table demonstrates the highly conserved antigenic nature of the nucleoprotein that was identified in most IFA-positive cheetah sera with all domestic cat coronaviruses in this study. Less conserved were the membrane antigens, which were recognized by 20 to 85% of the serum samples, depending upon the virus isolate. These data suggest that African cheetahs have been exposed to several immunologically different strains of coronaviruses.

Comparison of cheetah coronavirus isolate to domestic cat coronaviruses. A feline coronavirus was isolated from a cheetah from Oregon which perished from FIP (7). The specificity of serum samples from several IFA-positive domestic cats and cheetahs were tested by Western blotting for reactivity to the cheetah isolate designated AJUCV-1 (8). Both the cheetah and domestic cat sera detected nucleocapsid proteins of AJUCV-1, FIPV 79-1146, and FIPV UCD-1 (Fig. 4). Reactivity of the sera from both species to the membrane protein was variable, indicating antigenic heterogeneity within the viral populations that infect these animals.

DISCUSSION

We have presented a serological survey of the incidence and pattern of exposure to feline coronavirus in captive and free-ranging cheetahs in North America, Europe, and Africa. In addition we have summarized the results of a retrospective serum survey of the FIP epizootic that began in 1982 at a cheetah-breeding facility in Winston, Oreg. Because of the shortage of epidemiological studies of this disease in cheetahs and in light of the danger of too-strict extrapolation from disease progression in the domestic cat, it seems important to understand the lessons of this epizootic as a basis for interpreting incipient outbreaks in cheetah populations in captivity and in their natural range.

The Oregon outbreak occurred with the arrival at the facility in 1982 of an infected animal from another park. Before 1982 all of the cheetahs in the park were coronavirus seronegative. Within 8 months all cheetahs in the park

TABLE 2. Feline coronavirus antibody titers and EM results in North American zoologic facilities in 1986 and 1987

Serum result and facility no.	Year	Total no. of cheetahs at facility	No. of cheetahs with the following coronavirus IFA titers (X10)					EM screen of feces	
			≤25	25	100–125	400–625	≥1,600	No. positive/ no. tested	% Positive
Seronegative									
1	1986	10	9	0	0	0	0	0/9	0
	1987		2	0	0	0	0	ND[a]	
2	1986	14	7	0	0	0	0	1/8	13
	1987		11	0	0	0	0	ND	
3	1987	3	3	0	0	0	0	ND	
4	1986	9	9	0	0	0	0	9/9	100
Seropositive									
5	1986	4	3	1	0	0	0	3/3	100
	1987		1	0	0	5	3	ND	
6	1986	20	3	1	1	0	0	2/6	33
	1987		7	6	2	0	6	ND	
7	1986	5	2	1	0	1	1	3/5	60
	1987		0	0	0	1	4	ND	
8	1986	22	10	0	5	3	1	1/22	5
	1987	39	17	5	5	9	3	ND	
Indeterminant									
9	1986	3	3	0	0	0	0		
10	1986	5	5	0	0	0	0		
11	1986	2	3	1	0	0	0	0/1	0
12	1987	1	1	0	0	0	0		
13	1986	1	1	1	0	0	0	1/2	50
14	1986	17	1	0	0	0	0		
15	1986	4	2	0	0	0	2	1/2	50
16	1986	2	0	2	0	0	0		
17	1986	1	0	0	0	0	1	1/1	100
18	1986	5	0	0	0	2	3		
19	1986	4	1	2	0	0	1		
20	1986	7	0	0	0	1	0		

[a] ND, Not determined.

seroconverted, and after 4 years 60% had died of FIP-related disease. Age of infection was a significant risk factor, with infants (0 to 5 months of age) being at greater risk. The median time from seroconversion (as a marker of exposure) to death was 6 to 12 months. Interestingly, there was no obvious difference between the temporal pattern of feline coronavirus serum antibody titers in cheetahs who died versus those who survived (compare Fig. 1a and b).

TABLE 3. Western blot results comparing antigens of four strains of feline coronavirus in reaction with sera from 20 cheetahs[a]

Feline coronavirus isolate	Sequelae in domestic cats	No. positive (%) on Western blot of[b]:	
		Membrane antigen (29 kDa)	Nucleoprotein (45 kDa)
WSU 79-1683 (FECV)	Enteritis, non-FIP; high morbidity, low mortality	17 (85)	19 (95)
UCD-1 (FIPV)	FIP dose-related virulence; high morbidity, high mortality	4 (20)	18 (90)
WSU 79-1146 (FIPV)	FIP very virulent, low dose; high morbidity, high mortality	13 (65)	20 (100)
NOR-15 (prototype strain)	FIP very virulent, low dose; high morbidity, high mortality	14 (70)	18 (90)

[a] Twenty cheetah sera (Table 1) with coronavirus IFA titers of >25.
[b] Results indicate numbers and percentages of cheetah sera which identified the nucleoprotein and membrane antigens in Fig. 3.

The reasons why some cheetahs survived while others perished are not clear, but, based on precedence from other coronavirus and retroviral epizootics, there are three possible explanations (22, 24, 36). First, there is genetic variation in virus isolates that produce different clinical results. This seems to be the case in the domestic cat viruses, which range from extremely virulent to subclinical (Table 3). Nevertheless, despite the isolation of clinically distinct domestic cat coronaviruses, it has not been possible to develop type-specific immunological reagents that discriminate between them (11–14). There is currently no direct evidence for functional heterogeneity of cheetah coronaviruses; however, the discordance of coronaviruslike particles detected by EM and the results of IFA and Western blot analyses (Table 2) is consistent with appreciable antigenic diversity among cheetah coronaviruses. Second, there could be genetic differences in the host cheetahs that influence the pathology. This is possible despite our earlier observation of the reduced genetic diversity of cheetahs (23–26), since those results showed reduced (10 to 100-fold) diversity but not complete homozygosity at all loci. In fact, we have observed limited heterozygosity in cheetahs by using molecular clones of one gene involved in immune surveillance, the major histocompatibility complex (40a). Third, FIP pathology could involve

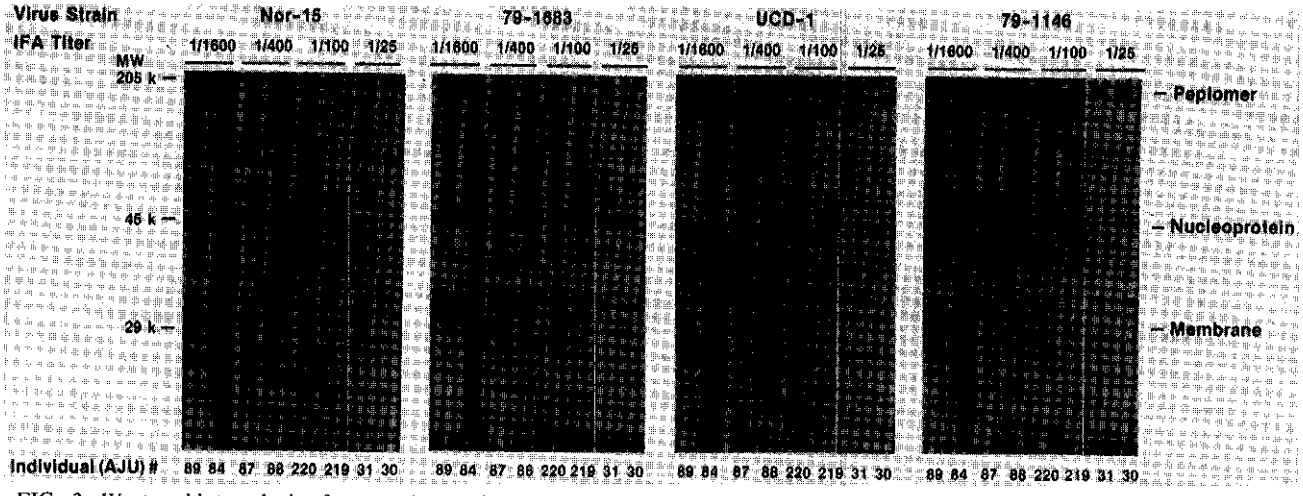

FIG. 3. Western blot analysis of coronavirus antigens with selected cheetah sera. Four feline coronavirus strains, FIPV NOR-15, FECV WSU 79-1683, FIPV UCD-1, and WSU FIPV 79-1146 (Table 3), were reacted with eight cheetah serum samples with IFA titers ranging from 1:25 to 1:1,600. Cheetahs AJU 84, 87, 88, and 89 were from Wildlife Safari; AJU 219 and 220 were captive animals in Kenya; and AJU 30 and 31 were from De Wildt Cheetah Research Center in South Africa. The molecular weight markers are listed on the left, and the main coronaviral protein regions are listed on the right.

a cofactor such as a secondary synergistic virus or possibly a stochastic (somatic or environmental) event to cause pathology. For example, FIP has been suggested to progress more rapidly and with more serious consequences in cats concurrently infected with feline leukemia virus (27) or with feline immunodeficiency virus (40).

In hopes of resolving some of these possibilities, we have used three different methods for detecting feline coronavirus in cheetahs: IFA assay, Western blots, and EM screening for coronaviruslike particles in feces. The IFA method seems rather verifiable with clinical disease, based upon the Oregon epizootic and disease outbreaks in several other facilities (19). Further, the IFA assay and Western blots show excellent correlation insofar as every IFA-negative serum was also negative on Western blots and all IFA-positive sera detected FIPV nucleoproteins, although there was some variation in detection of the membrane protein. Notable shifts in the molecular weight of the nucleoproteins observed here (Fig. 3 and 4) may represent strain variations between viral isolates but more likely reflect developmental processing of nucleocapsid proteins previously reported for feline coronavirus (36).

There was not a good correlation with the EM detection of fecal coronaviruslike particles and the IFA assay. It is

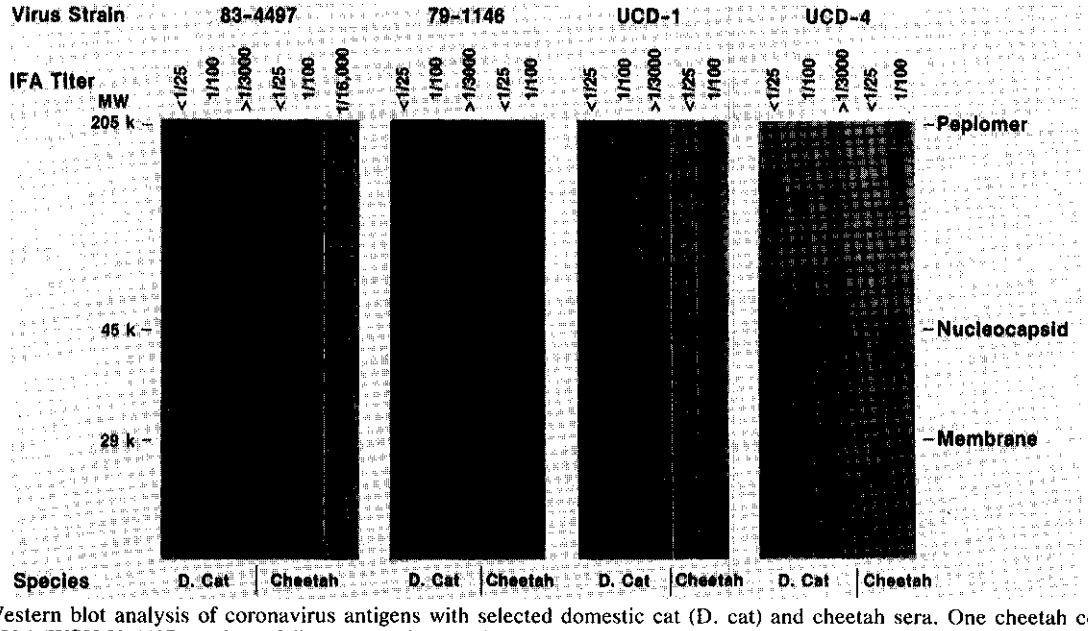

FIG. 4. Western blot analysis of coronavirus antigens with selected domestic cat (D. cat) and cheetah sera. One cheetah coronavirus strain, AJUCV-1 (WSU 83-4497), and two feline coronavirus strains, FIPV WSU 79-1146 and FIPV UCD-1, were reacted with sera with IFA titers ranging from negative (<1:25) to positive (>1:25 to 1:16,000). The molecular weight markers are listed on the left, and the main coronaviral protein regions are listed on the right.

possible that this discordance would result from an acquired infection before the development of an immune response. Based upon the well-known existence of antigenic drift in RNA viruses (41) and in coronaviruses (4, 17, 32, 39), it is also likely that these particles may represent viruses that are morphologically similar to but antigenically distinct from the recognized strains of FIPV and FECV (20, 36). The discordance between IFA serology results versus EM coronavirus screening in cheetahs makes it difficult to interpret the EM results as a diagnostic parameter for cheetahs.

The observations of the Oregon outbreak plus the results of the IFA serum survey for free-ranging and captive cheetahs have suggested the following conclusions, which we recommend for consideration of this pathogen in managed cheetah populations.

(i) Pathological FIPV is highly infectious and spreads rapidly among cheetahs when physical contact occurs, probably through exchange of excretory material and/or secretions (34).

(ii) Pathological FIP is a dynamic disease process that is correlated with continuous, measurable, and often increasing titers to feline coronavirus.

(iii) Occasional low antibody titers have been observed in several populations, including free-ranging animals (Table 1 and 2), which could signal an incipient disease epizootic but may also reflect infection with a nonpathological but antigenically related coronavirus. The confirmation of a clinical epizootic would require time points showing increasing numbers and higher titers (as in facilities 5 through 8) plus morbidity. Facilities with increasing titers but no disease (facilities 5 through 8 in Table 2) may prove particularly interesting, because they raise the possibility of clinical heterogeneity of virus isolates between different facilities. Long-term monitoring of these facilities to confirm this hypothesis is desirable.

The present results indicate that coronavirus infections are occurring in captive and free-ranging cheetah populations, as has been reported for domestic cats (1, 16, 27). The factors that appear to be important with the occurrence of FIP in a population of cats are a combination of a virulent strain of virus together with a susceptible group of cats (1, 16, 27). The cheetah has been reported as being unusually vulnerable to FIPV, based on epizootics that have occurred in the United States, Canada, Ireland, Namibia-Southwest Africa, The Netherlands, and Japan (9, 18, 19, 23). The results of the study reported herein indicated that the exposure rate to feline coronavirus, or a closely related coronavirus, is similar to that reported for domestic cats. Until further information is reported concerning coronavirus infections in exotic cats, the management of cheetahs should follow basic guidelines for control of infectious diseases, such as segregation of seropositive animals from seronegative animals, quarantine before and after arrival at the zoologic facility, and regular monitoring by serology (8).

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