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Abstract: Clinical and diagnostic features of the 1982-83 epizootic of Feline Infectious Peritonitis (FIP) and the effects on the cheetah population of Wildlife Safari in Oregon are presented in tabular format. Test procedures and results are discussed, and photographs of affected cheetahs are included. The results of serologic testing of the survivors of the epizootic, as well as testing of new additions to the colony, were presented in a companion article.

## FELINE PRACTICE — EXOTIC MEDICINE

Clinical and diagnostic features of the 1982-83 epizootic of Feline Infectious Peritonitis (FIP) and the effects on the cheetah population of Wildlife Safari in Oregon are presented in tabular format. Test procedures and results are discussed, and photographs of affected cheetahs are included. The results of serologic testing of the survivors of the epizootic, as well as testing of new additions to the colony, were presented in a companion article published in Feline Practice (16[2]:13-16, March-April, 1986).

### CLINICAL AND DIAGNOSTIC FEATURES

## Feline Coronavirus Infections of Cheetahs

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### Introduction

During 1982-83 there was an epizootic of Feline Infectious Peritonitis (FIP) in a captive cheetah population in the United States.<sup>1</sup> The epizootic was considered to be one of the most severe disease outbreaks of FIP recorded in either domestic or zoologic cats.<sup>1</sup> Over the period of 14 months, 13 cheetahs died, including three adults. During the intervening 2 years, the surviving population has been closely monitored by attending veterinarians and animal care personnel. In addition to maintaining a close surveillance on the health of the cheetahs, the population has been routinely tested for the presence of feline coronavirus antibodies and fecal shedding of coronaviruses by electron microscopy. The purposes of this report are to present the clinical and diagnostic features

of the original epizootic and the outcome of survivors. The results of the serologic testing of the survivors, as well as the new additions to the colony, were presented in an accompanying article.<sup>2</sup>

### Materials and Methods

#### ANIMALS

The cheetahs studied were part of a large zoologic facility located in an inland valley in southwest Oregon. The facility consists of 650 acres of which 200 acres are developed and fenced. Five groups of cheetahs were monitored during the 1982-83 epizootic. The original group designations will be maintained for clarity throughout this report.<sup>1</sup> They included:

- Group A, representing the free-roaming cheetahs in the drive-through area of the

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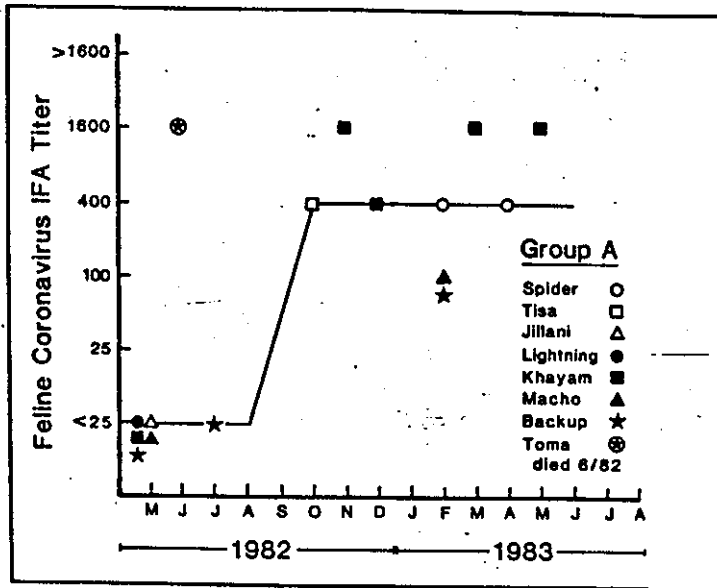


Fig. 1a — Serological results of cheetahs in drive-through compound (Group A).

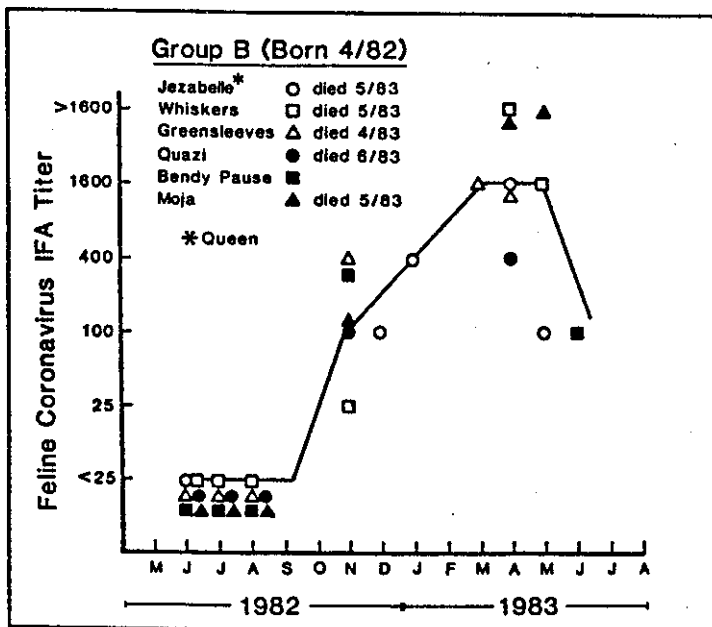


Fig. 1b — Serological results of cheetahs in litter born April, 1982 (Group B).

park

- Groups B, C and D, representing the cheetahs located in maternity pens
- Group E representing adult cheetahs in the breeding compound.

CLINICAL SIGNS

The cheetahs were observed on a daily basis for appetite, mobility and, where appropriate, interaction with companion cheetahs. Records were maintained on occurrence of lethargy, inappetence and diarrhea.

SEROLOGY

Antibody to the feline coronavirus was determined by the Indirect Immunofluorescent Antibody (IFA) test as previously described.<sup>1</sup> Briefly, Crandell Feline Kidney (CrFK) cells were inoculated simultaneously with canine coronavirus (1-71 strain) in multi-chambered slides (Bellco, Vineland, New Jersey). The virus-infected cells served as the substrate for the IFA test. Serum samples were routinely diluted in four-fold serial dilutions beginning at 1:25 and proceeding to 1:1600. Rabbit anti-feline IgG conjugated to Fluorescein Isothiocyanate (FITC:Cappel Labs, Cochranville, Pennsylvania) was diluted 1:20 for use in the test. Serum antibody titers were expressed as the reciprocal of the highest dilution resulting in positive fluorescence.

VIROLOGY

Tissue homogenates (10%) and fecal suspensions in cell culture medium were inoculated onto 1-day-old monolayer cultures of CrFK cells. The cells were observed for the presence of Cytopathogenic Effect (CPE). Suspected viral isolates were further tested in CrFK cells for the presence of inclusion bodies by Hematoxylin and Eosin (H&E) staining. Cytopathogenic agents were also tested by IFA for the presence of coronavirus antigens. The immunofluorescence system consisted of rabbit anti-FIPV (NOR-15 strain) and goat anti-rabbit IgG FITC (Cappel Labs).

PATHOLOGY

Sections of liver, kidney, spleen, lymph nodes, thymus, stomach and gastrointestinal tract were preserved in buffered formalin and submitted to the diagnostic laboratory for histopathologic analysis. The tissues were processed as previously described.<sup>1</sup>

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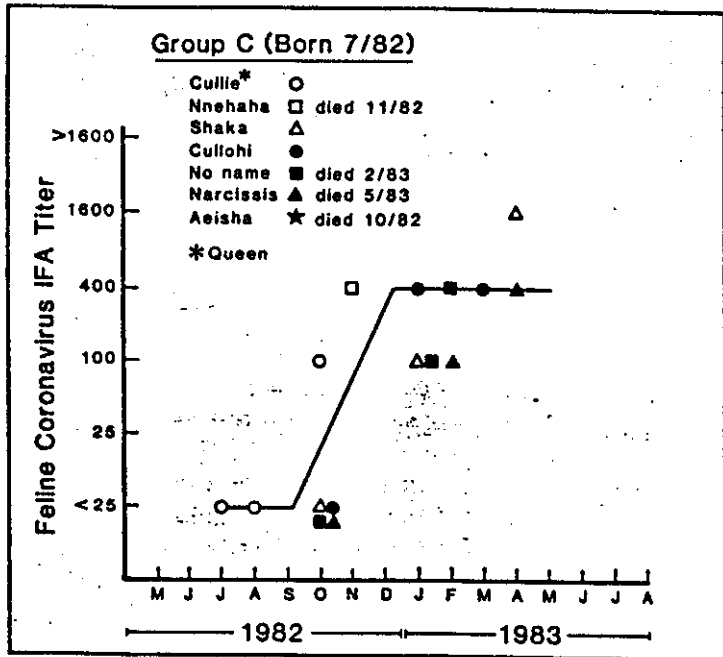


Fig. 1c — Serological results of cheetahs in litter born July, 1982 (Group C).

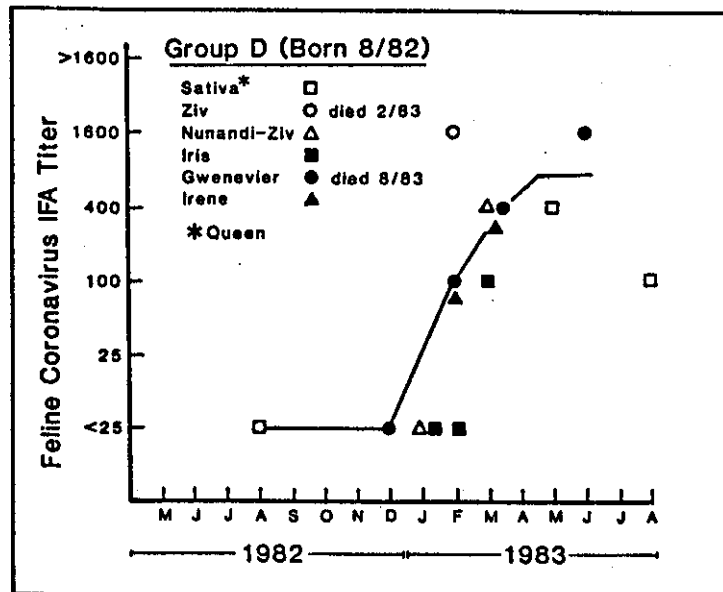


Fig. 1d — Serological results of cheetahs in litter born August, 1982 (Group D).

**Results**

**CLINICAL AND SEROLOGICAL OBSERVATIONS OF THE ORIGINAL EPIZOOTIC**

Five groups of cheetahs were involved in

the analyses. The first group (Group A) represented the free-roaming cats in the drive-through area of the park. The antibody titers to feline coronavirus are shown in Figure 1a. The resident cheetahs were seronegative prior to May, 1982. During this time a cheetah (Toma) was acquired from another zoologic facility for breeding purposes and was the first case of FIP at the park in Oregon. Toma died in June, 1982. An in-depth study of that initial case was the subject of another report.<sup>3</sup> The next groups of cats (Groups B, C and D) were those located in the maternity pens (Figs. 1b, 1c and 1d). The fifth group (Group E) (Fig. 1e) represented the cheetahs in the breeding colony.

After the initial case of FIP was observed in June, 1982, there was a 10-month period in which the cats were experiencing intermittent diarrhea, but there were no deaths attributable to FIP. In April of 1983, a litter of five cheetah cubs (Group B) began to show clinical signs of FIP characterized by lethargy, anorexia and weight loss. The cubs were noted to be depressed with swollen abdomens (Fig. 2). The final stages of FIP observed were sunken eyes (Fig. 3) and erosions around the nostrils and lips (Fig. 4).

In several cheetahs there were depigmented areas above the incisors as a result of current ulcerative glossitis (Fig. 5). The queen of this litter also became ill and died in May, 1983. Retrospective serologic studies indicated that this litter was exposed to feline coronavirus in the fall of 1982, with the first seropositive cubs noted in November of that year (Fig. 1a). The lone survivor of this litter (Bendy Pause) maintained a titer of 1:100.

Two additional groups (Groups C and D) within the maternity section were also observed during the FIP epizootic. The Group C litter (Fig. 1c) started showing clinical signs of FIP in the fall of 1982 with four of six cubs succumbing by May of 1983. Only two cubs out of the Group D litter (Fig. 1d) died. The infection was detected serologically early in 1983. The last death attributed to FIP in the 1982-83 epizootic (Gwenevier) was in August, 1983.

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Fig. 2 — Cheetah cub, Moja: depressed and swollen abdomen.



Fig. 3 — Cheetah cub Moja: sunken eyes, punched down lower eyelids and moist nose (typical signs of final stage of FIP in the cheetah).



Fig. 4 — Cheetah cub, Whiskers: moribund with erosions on the nostrils and lips.

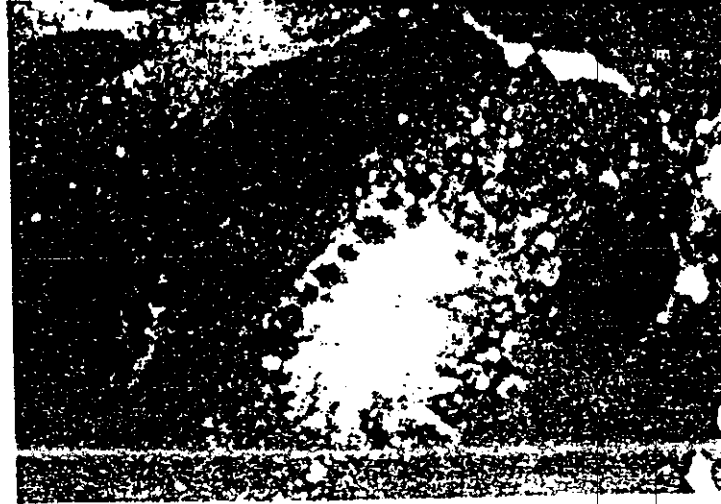


Fig. 5 — Cheetah cub Moja: depigmented area above incisors, extremely pale color indicating anemia.

Fig. 6 — Histopathologic section of spleen from cheetah with FIP. Note distorted splenic capsule resulting from a thick plaque of fibrin and cellular debris (H&E stain, original mag 40X).



Fig. 7 — Immunofluorescence of CrFK cells infected with virus isolated from cheetah with FIP. Stained with rabbit antisera to feline coronavirus, NOR 15 (indirect strain).



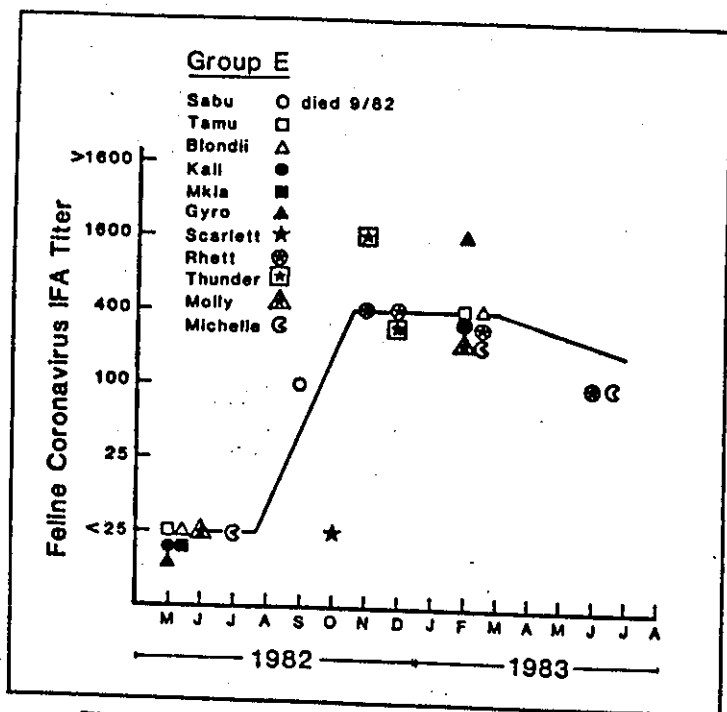


Fig. 1e — Serological results of cheetahs in breeding colony (Group E).

The last group of cheetahs tested serologically for feline coronavirus were those located in the breeding compound. Of the 11 animals in this group (Group E), all had diarrheal episodes ranging from acute to chronic. All the cats were seronegative in the spring of 1982 (Fig. 1e). However, by the fall of that year all except one cat (Scarlett) seroconverted to feline coronavirus. One cat (Sabu) died in this group in September, 1982. Death was attributed to chronic renal disease and enteritis. The cat had been in contact with Toma, the first cat to show signs of FIP, 3 months earlier.

#### PATHOLOGICAL OBSERVATIONS

Tissues from the cheetahs submitted for microscopic examination contained lesions characteristic of FIP. Multifocal necrosis attributable to FIP was present throughout many organs including the liver, kidneys, pancreas, spleen, lymph nodes and thymus. Necrotic areas were characterized by karyorrhexis, karyolysis, cytolysis and infiltration by lymphocytes and macrophages. Inclusion

bodies were not seen. Fibronectic plaques were present in pleural and peritoneal surfaces of many organs (Fig. 6) and were confluent with areas of necrosis in underlying parenchyma. Necrotic foci containing lymphocytic infiltrates, macrophages and fewer neutrophils were also present in mesenteric fat and within the *tunica muscularis* of the small and large bowel. Lymphoid aggregates in the spleen and lymph nodes were depleted. Necrotic foci in these organs commonly contained areas of lympholysis.

Several other lesions were noted in addition to changes attributed to FIP. The gastric mucosa of four animals contained superficial erosions of mucosal epithelium. In most cases, the changes were relatively mild with hyperemia of superficial mucosal vessels, mild focal hemorrhages and mild fibrosis of the interstitium. A gastric ulcer was present in one animal. Epithelium within gastric pits was generally intact, although fibroblastic response bridged gastric pits in some areas. A moderate interstitial infiltrate of lymphocytes, plasma cells and macrophages was associated with areas of erosion.

Significant accumulation of yellow crystalline material was evident within the renal tubules of two animals. The crystals were birefringent with polarized light and were identified by microincineration as calcium oxalate. There was moderate, segmental dilation of renal tubules and mild interstitial fibrosis in kidney sections which contained crystals.

#### VIROLOGICAL OBSERVATIONS

Samples from tissues obtained at necropsy and from swabs and fecal specimens collected from clinically ill cheetahs were processed for virus isolation as previously described.<sup>1</sup> Briefly, all samples were inoculated into 1-day-old monolayer cultures of CrFK cells. The cultures were observed daily for the presence of CPE. Those cultures showing CPE were passaged by routine trypsinization, as well as by freezing and thawing and reinoculation onto freshly prepared CrFK cells. Three cytopathogenic agents were isolated from the tissue homogenates. The

Continued

**TABLE 1**  
**Outcome of Survivors of the 1982-83 FIP Epizootic in Captive Cheetahs**

Group	Animal Identity	Age (years)	Outcome*
A (Drive-through compound)	Spider	7	Died — renal failure
	Tisa	8	Alive — transferred
	Jillani	9	Alive — transferred
	Lightning	16	Alive
	Khayam	9	Alive
	Macho	9	Alive
	Backup	9	Alive
B (Litter born 4/82)	Bendy Pause	3	Alive
C (Litter born 7/82)	Cullie (queen)	9	Died — FIP
	Shaka	3	Died — FIP and pyothorax
	Cullohi	1	Died — FIP
D (Litter born 8/82)	Sativa (queen)	10	Alive
	Nunandi Ziv	2	Died — FIP
	Iris	2	Died — FIP
	Irene	3	Alive
	Tamu	4	Alive
E (Breeding colony)	Blondii	4	Alive
	Kali	4	Alive
	Mkia	4	Alive
	Gyro	4	Alive
	Scarlett	8	Died — FIP
	Rhett	8	Alive — transferred
	Thunder	6	Died — Liver dysfunction-enteritis
	Molly	14	Died — renal failure
	Michelle	9	Alive

\* Disposition of cheetahs as of September 1985.

viruses were highly cell-associated and were not readily passaged by freezing and thawing. Monolayer cultures of infected cells were stained by immunofluorescent techniques. The isolates were reactive to anti-FIP virus (NOR-15) serum prepared in rabbits (Fig. 7). Further identification of these isolates is in progress.

**OUTCOME OF SURVIVORS**

Since 1983, nine additional cheetahs from the original five groups have died. Table 1 summarizes the list of surviving cheetahs and their outcome. Of the seven surviving animals in Group A, one died from renal

failure and two were transferred to another zoological park. Bendy Pause was the only survivor of Group B and is currently considered to be in good condition. Of the three original survivors in Group C all have succumbed to FIP in the intervening 2 years, including the queen, Cullie.

Group D is currently represented by two cheetahs, having lost two littermates in 1984 to FIP. Nunandi Ziv, the first cheetah to die, was noted to be losing weight for 2 to 3 weeks with an increase in White Blood Cells (WBC) and total bilirubin. The histologic diagnosis was pyogranulomatous peritonitis and pleuritis. The second cheetah to die from this

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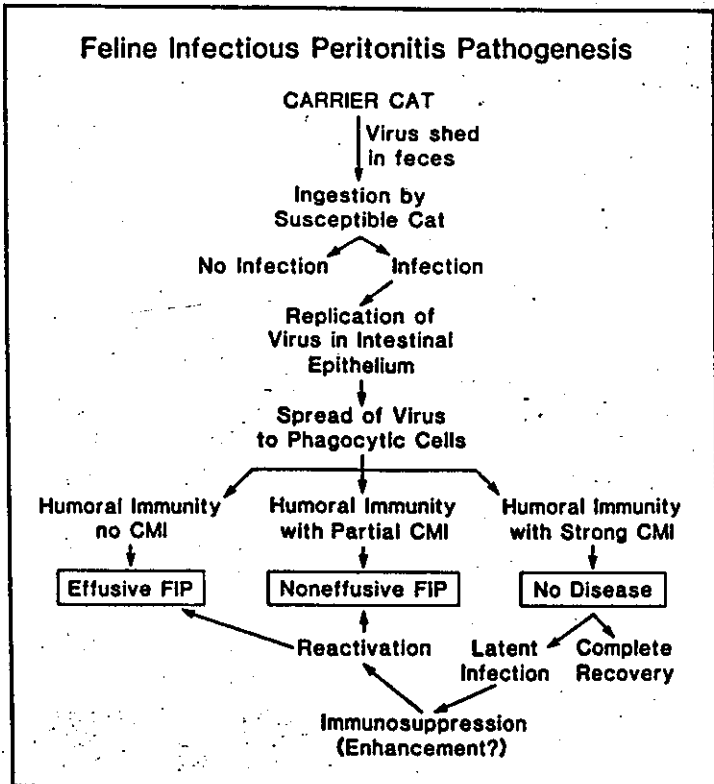


Fig. 8 — Proposed pathogenesis of feline infectious peritonitis virus modified from Pedersen, *et al.*<sup>1,4,5</sup>

group was Iris. She was noted to be lethargic for several weeks, with initial weakness in her hind limbs. She eventually became paralyzed in all four limbs and succumbed from the neurologic form of FIP. Both of the cheetahs had feline coronavirus IFA titers of > 1:1600 just prior to death.

Group E cheetahs have had three deaths — Scarlett to classical FIP, Thunder to liver dysfunction-enteritis and Molly to renal failure.

### Discussion

During the intervening 2 years since the 1982-83 FIP epizootic, six more cheetahs have died due to FIP, adding to the severity of the outbreak and reemphasizing the importance of understanding the pathogenesis of the feline coronaviruses. Data are emerging which indicate the importance of the host immune responsiveness to the virus.

Pedersen postulated the importance of Cell-Mediated Immunity (CMI) in protecting the cat against systemic forms of feline coronavirus infection, commonly referred to as FIP, or feline coronaviral vasculitis (Fig. 8).<sup>4,6</sup> Recent studies have indicated that the thymus-derived lymphocytes (T cells) are important in defending against FIP.<sup>7,8</sup> These results serve to explain why FIP is more severe in cats with concurrent Feline Leukemia Virus (FeLV) infection, which has been shown to have immunosuppressive effects upon the T cells.<sup>5</sup> Although the severity of feline coronavirus infection in the cheetahs could not be related to concurrent FeLV infection since the cheetahs had been tested as being FeLV antigen negative (Leukassay F: Pitman-Moore), it could be related to a genetic defect in T cell responsiveness. Recent reports by O'Brien *et al.* have shown that the cheetah is genetically monomorphic and lacks the ability for rapid skin graft rejection.<sup>9,10</sup> These observations have led to the speculation that the cheetah may have a deficiency in its CMI response.<sup>1,10</sup>

It would now appear that the host's immune response to the feline coronavirus is one of the most important factors in determining whether the infected cat will develop fatal coronaviral vasculitis.<sup>11,13</sup> However, two other factors need to be considered — the strain of virus and the route of virus entry into the cat's body.<sup>4,5,12</sup>

Several strains of feline coronavirus have now been isolated and, depending upon their pathogenesis, they are classified as either feline enteric coronaviruses (localized to the alimentary tract) or feline infectious peritonitis (systemic spread to body organs).<sup>12,14</sup> Clear differentiation of these strains, either by electron microscopy or current serologic tests, is not currently possible.<sup>15</sup> However, serology can be used to monitor exposure to coronavirus and allows for a prognosis to be made depending on how elevated the antibody titer becomes.<sup>1</sup> This was well demonstrated by the serological responses of the cheetahs during the 1982-83 epizootic (Figs. 1a-1e).

The route of FIP virus entry has not been

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well studied in terms of feline coronavirus pathogenesis. Some FIP virus isolates will cause only an enteritis when inoculated orally into cats.<sup>16</sup> Comparatively, the antigenically related coronavirus of dogs is also considered to be a localized infection when acquired by the oral route. However, in 1983 during the use of a modified live canine coronavirus vaccine, the virus was inoculated intramuscularly, resulting in clinical signs that were similar to FIP in cats in a high percentage of inoculated dogs.<sup>17</sup> The reason behind this unusual reaction was considered to be the immunosuppressive effect of other modified live viruses administered at the same time. The potential for feline coronavirus being more virulent if introduced by cat bites, licking open wounds, etc., needs to be verified.

The high mortality rate (19 cheetahs) and length of time of the FIP outbreak (3 years) in the captive cheetah population indicate that further studies are essential to determine how long and in what form the feline coronaviruses are being shed (free virus, virus-antibody complexes, etc.). It is also important to determine what measures can be taken in the future to control infection and minimize disease in this endangered species.<sup>18,19</sup> ■

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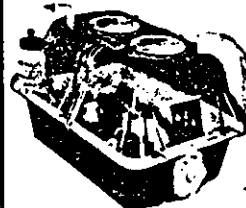
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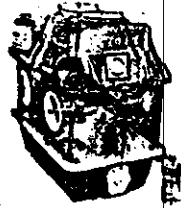
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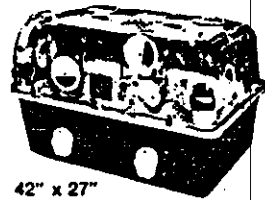


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