Response of Mink, Skunk, Red Fox and Raccoon to Inoculation with Mink Virus Enteritis, Feline Panleukopenia and Canine Parvovirus and Prevalence of Antibody to Parvovirus in Wild Carnivores in Ontario

I.K. Barker, R.C. Povey and D.R. Voigt*

ABSTRACT

Mink virus enteritis, feline panleukopenia and canine parvovirus-2 were inoculated separately into groups of raccoon, mink, red fox and striped skunk. Raccoons were highly susceptible to mink virus enteritis and feline panleukopenia, with animals developing clinical illness, and several dying within six to ten days of inoculation with lesions typical of parvovirus infection. Both viruses were shed in high titre in the feces of infected raccoons, and high antibody titres were stimulated. Raccoons inoculated with canine parvovirus-2 showed no signs; shedding of virus was sporadic though moderate titres of antibody developed. Mink inoculated with mink virus enteritis and feline panleukopenia developed signs and lesions of early parvovirus infection. No signs or significant lesions followed canine parvovirus-2 inoculation. Shedding of virus was heavy (mink virus enteritis) or sporadic (feline panleukopenia and canine parvovirus-2), though good serological responses were elicited to all three viruses. Red fox showed no signs of infection, shed all three viruses only sporadically, and the serological response was strong only to feline panleukopenia. Skunks developed low antibody titres, but no signs, and did not shed virus. Antibody to parvovirus was found in 79.2% of 144 wild red foxes; 22.3% of 112 wild raccoons; 1.3% of 157 wild skunks and 6/7 coyotes in southern Ontario. The likely significance of these viruses to wild and captive individuals and populations of these carnivores is discussed.

Key Words: Parvovirus, mink virus enteritis, feline panleukopenia, canine parvovirus-2, raccoon (*Procyon lotor*), red fox (*Vulpes vulpes*), striped skunk (*Mephitis mephitis*), mink (*Mustela vison*), coyote (*Canis latrans*), serological survey.

RÉSUMÉ

Cette expérience consistait à inoculer séparément, à des groupes de ratons laveurs, de visons, de renards roux et de mouffettes rayées, les virus de l'entérite virale du vison et de la panleucopénie féline, ainsi que le parvovirus #2 canin.

Les ratons laveurs s'avérèrent très réceptifs aux virus de l'entérite virale du vison et de la panleucopénie féline; ils manifestèrent des signes cliniques et plusieurs moururent au bout de six à dix jours après leur inoculation; ces derniers affichaient des lésions typiques d'une infection à parvovirus. Les ratons laveurs infectés éliminèrent dans leurs fèces une forte quantité des deux virus; ils développèrent aussi des titres élevés d'anticorps. Ceux auxquels on avait inoculé le parvovirus #2 canin ne manifestèrent pas de signes cliniques; ils ne l'éliminèrent dans leurs fèces que de façon sporadique, mais ils développèrent quand même des titres modérés d'anticorps.

Les visons auxquels on inocula le virus de l'entérite virale du vison et celui de la panleucopénie féline manifestèrent des signes cliniques et développèrent des lésions compatibles avec une infection précoce à parvovirus. Leur inoculation avec le parvovirus #2 canin ne provoqua ni signes cliniques ni lésions. Ils éliminèrent dans leurs fèces une forte quantité du virus de l'entérite virale du vison; quant à celui de la panleucopénie féline et au parvovirus #2 canin, ils n'en éliminèrent que de façon sporadique; ils développèrent toutefois de bons titres d'anticorps à l'endroit de ces trois virus.

Les renards roux ne manifestèrent aucun signe d'infection et l'élimination fécale des trois virus se révéla sporadique. Ils ne développèrent une forte réaction sérologique qu'à l'endroit

*Department of Pathology (Barker), Departments of Clinical Studies and Veterinary Microbiology and Immunology (Povey), Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1 and Wildlife Branch, Research Section, Ontario Ministry of Natural Resources, Box 50, Maple, Ontario L0J 1E0 (Voigt).

Ontario Ministry of Natural Resources, Wildlife Research, Contribution No. 82-06.

Submitted May 14, 1982.

du virus de la panleucopénie féline.

Les mouffettes rayées développèrent de faibles taux d'anticorps; elles ne manifestèrent pas de signes cliniques et n'éliminèrent pas de virus dans leurs fèces.

La recherche d'anticorps à l'endroit du parvovirus, dans le sérum d'animaux vivant à l'état sauvage, au sud de l'Ontario, donna les résultats suivants: 79,2% de 144 renards roux; 22,3% de 112 ratons laveurs; 1,3% de 157 mouffettes rayées; six coyotes, sur un total de sept. Les auteurs commentent la signification probable de ces virus pour ces carnivores, tant pour les individus qui vivent à l'état sauvage ou en captivité que pour leur population, considérée dans son ensemble.

Mots clés: parvovirus, entérite virale du vison, panleucopénie féline, parvovirus #2 canin, raton laveur (*Procyon lotor*), renard roux (*Vulpes vulpes*), mouffette rayée (*Mephitis mephitis*), vison (*Mustela vison*), coyote (*Canis latrans*), enquête sérologique.

INTRODUCTION

Feline infectious enteritis or feline panleukopenia has been recognized as a disease entity for over 50 years and was well described clinically and pathologically by 1939 (1). It is considered that most, if not all, members of the Felidae are susceptible (2, 3) though natural disease has not been recognized in free-living wild felids (4). The first *in vitro* cultivation of feline panleukopenia virus (FPV) was achieved with material from a leopard (5) and the virus has been characterized as a parvovirus (6).

Mink virus enteritis (MVE) was first recognized near Fort William, Ontario in 1947, and has occurred wherever mink (*Mustela vison*) are ranched (7). The close relationship of FPV and MVE was recognized early (8) and subsequent studies have demonstrated that MVE is a parvovirus closely related to, if not identical with, FPV (6, 9, 10). Reports of infection of cats with MVE (8, 11, 12) and mink with FPV (11, 12, 13) indicated that infection of the heterologous host occurred, producing signs of disease and lesions in some cases. However, disease was more difficult to induce in the heterologous host and the viruses have been distinguished by their relative pathogenicity in cats and mink (7).

In 1978 a disease of dogs (*Canis familiaris*) pathologically similar to FPV and MVE was newly recognized simultaneously worldwide (14, 15, 16). This disease was demonstrated to be associated with a canine parvovirus (17, 18, 19) distinct from a previously reported parvovirus of dogs (20), and best designated CPV-2. Subsequently disease associated with CPV-2 was seen in captive maned wolves (*Chrysocyon brachyuris*) (21, 22); bush dog (*Speothos venaticus*), crab eating fox (*Cerdocyon thous*) (22); and coyote (*Canis latrans*) (23). Canine parvovirus-2 is antigenically closely related to FPV (24) but distinguishable by serum neutralization (25) or agar-gel precipitation (26). By restriction site mapping of the viral genome, a close relationship between CPV-2 and MVE has been demonstrated (27). The restriction enzyme analysis did show differences between CPV-2 and MVE at 11 sites in regions coding for viral capsid proteins. This work has now been extended with the finding of similar genome differences between FPV and CPV (28).

Diseases clinically and pathologically resembling FPV have been occasionally reported from other carnivores. These have usually been unconfirmed by virus isolation. Cases have involved Arctic Fox (*Alopex lagopus*) (29), viverrid civet cats (30), procyonids including the coatimundi (31) and most commonly the raccoon (*Procyon lotor*). In raccoons the disease has usually been considered to be due to FPV (2, 3, 32), since tissues from infected raccoons caused FPV-like disease in young cats (*Felis domesticus*) and the disease in raccoons was controlled by administering feline anti-FPV serum (32). However unpublished work (Alberts JO, Stutz D, Reynolds HA, Fritz TE. A transmissible enteritis of raccoon stimulating virus enteritis of mink. Unpublished Annual Progress Report 1959-60, Project 44-15-70-353. Mink Enteritis of Virus Origin, Dept. of Veterinary Research, Illinois Agricultural Research Station.) indicates the lethality for mink of infective intestine from raccoons affected by FPV-like disease and reports death of raccoons inoculated with MVE-infected mink tissue. Furthermore, a virus differing from FPV and resembling CPV-2 has been isolated from raccoons with parvovirus enteritis (33).

Because of the confusing and apparently overlapping host susceptibilities to FPV and MVE, the recent emergence of CPV-2, and the potential significance of these viruses to captive individuals or wild populations of economically, esthetically and ecologically significant carnivores, we undertook to study the susceptibility to these viruses of mink, striped skunk (*Mephitis mephitis*), red fox (*Vulpes vulpes*) and raccoon. In addition, a small serological survey was made for antibodies to parvoviruses in wild carnivores in southern Ontario, since no information was available on their prevalence in any wild populations.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Mink derived from the unvaccinated specific disease free (mink virus enteritis, canine distemper, aleutian disease) herd formerly maintained by the Ontario Veterinary College were used at between six months and 1.5 years of age. Skunks were obtained from a colony maintained by the Ontario Ministry of Natural Resources and the Ontario Veterinary College and ranged from two to six years of age. The younger animals were born in captivity but a few older animals were obtained originally from the wild. Raccoons and foxes, between eight weeks and seven months old, were trapped or col-

lected directly from the wild in southern Ontario.

Foxes, raccoons and skunks were held in stainless steel cat, dog or primate cages, appropriate to the size of the animal and the degree of security and ease of handling required. Mink were held in standard wire mesh cages. All animals were fed a commercial frozen mink food. Food was withheld on the day of inoculation and on the subsequent day, as this has been shown to increase the reliability of disease production in experimental challenge with CPV-2 (34).

Animals were housed in isolation rooms, which were entered through an anteroom following two changes of protective clothing and rubber boots, and after use of two sodium hypochlorite (2%) footbaths. Before each experiment cages and equipment were washed, rinsed with sodium hypochlorite solution and while wet the room and contents were fumigated for 18 hours with formaldehyde vapour. Separate isolation rooms were designated to hold animals inoculated with each of the three viruses, and each was served by a separate animal attendant.

Handling, inoculation of virus and withdrawal of blood samples were done while animals were anesthetized with a combination of ketamine hydrochloride and acepromazine maleate inoculated intramuscularly at dose rates of approximately 40 mg/kg and 1 mg/kg respectively. Animals obtained from the wild were handled as if potentially rabid.

VIRAL INOCULA

The inocula of MVE were 1:10 suspensions of spleen from infected mink (supplied by Connaught Laboratories, Willowdale, Ontario) suspended in phosphate buffered saline (PBS) at pH 7.2. Two separate inocula were prepared, with titres of $10^{4.2}$ and $10^{3.2}$ tissue culture mean infective doses ($TCID_{50}$)/mL.

Feline panleukopenia virus inocula were 1:10 suspensions in PBS of intestinal mucosal scrapings from a single cat which died from naturally acquired panleukopenia confirmed by histopathology. Two separate inocula of FPV were prepared containing $10^{3.0}$ and $10^{6.7}$ $TCID_{50}$ of virus/mL. Two cats each inoculated orally with $5\times$ $10^{3.0}$ $TCID_{50}$ of the virus developed clinical signs and seroconverted to high titre. One died nine days after inoculation (DAI) with microscopic lesions typical of panleukopenia.

Inocula of CPV-2 were prepared as approximately 1:10 suspensions in PBS of intestinal mucosa from dogs experimentally inoculated with CPV-2. Two inocula were prepared: the first had a titre of $10^{2.2}$ $TCID_{50}$/mL; CPV-2 could not be detected *in vitro* in the second inoculum, probably because of virus being complexed with antibody. Both inocula produced clinical signs and caused seroconversion to parvovirus in dogs inoculated during the course of other experiments.

Four or five mL of each inoculum was given into the stomach by intubation of each animal on day 0.

EXPERIMENTAL DESIGN

Following experimental infections two animals of each species from each of the inoculation groups (FPV, MVE, CPV) were killed 5 DAI and tissues were collected for microscopic examination for lesions. Three or four animals of each species were monitored for 12-21 days after inoculation with the three viruses. Animals were observed daily for clinical signs; feces were collected daily for subsequent attempts at virus isolation, and blood was collected by jugular venipuncture at intervals for serology. This protocol was followed with the mink and skunk, where batches of known seronegative (hemagglutination-inhibiting, HI, antibody titre $<1:8$) animals were available and could be inoculated simultaneously on the day they entered the isolation rooms (Day 0).

A modification of this protocol was necessary with foxes and raccoons, which were acquired sporadically and unpredictably. Each animal, of unknown serological status, was inoculated on the day of acquisition when it entered the appropriate isolation room. Serum collected at that time was titrated and any seropositive animals (HI titre $\geqslant 1:8$) were killed and removed from the experiment, usually by 3 DAI. In this manner, over a period of months, a series of experimental inoculations was accomplished. However, difficulty in obtaining sufficient seronegative fox pups during the two year study period prevented the full protocol from being fulfilled for infections of that species with CPV-2, FPV and MVE.

A summary of numbers of animals inoculated, size of inoculum and duration of observations for each experimental series is presented in Table I.

Animals killed by barbiturate overdose at 5 DAI were necropsied immediately and specimens of fundic stomach, duodenum, jejunum, ileum, colon, mesenteric lymph node, spleen, tonsil, bone marrow, thymus, heart, lung, liver and kidney were fixed in 10% neutral buffered formalin. Animals euthanized while moribund, or dying during the period of observation were similarly necropsied and tissues were collected. Wax-embedded tissues were sectioned at 6 μm and stained with hematoxylin and eosin for microscopic examination.

SEROLOGY

Serum was separated from clotted samples, heat inactivated at 56°C for 30 minutes and stored at -70°C until examined.

A microtitration hemagglutination-inhibition (HAI) test was used. The sera were adsorbed overnight at 4°C by adding 50 μL of each serum to 150 μL of African Green Monkey red cells (0.5% suspension) in two wells in the first row of a 96-well, round-bottomed microtiter plate. Following the adsorption, the supernatant serum (as a 1:4 dilution) was transferred to the next row of wells containing 50 μL of PBS (pH 7.2) using 50 μL microdiluters, and thus serially diluted across the plate. Appropriate positive and negative controls

were established. Four to eight hemagglutinating units of CPV-2 (strain Rae) were then added to each well. Plates were incubated for 1 h at 37°C and 50 μL of chilled 0.5% red cell suspension in PBS pH 7.2 (with 1% fetal bovine serum to aid settling) were added to all wells. Red cell controls were also established. Further incubation of the plates was at 4°C for two to 18 hours. A 50% end-point for inhibition of hemagglutination was determined for each serum by observation or extrapolation. The minimum detectable titre was 1:8.

VIRUS ISOLATION

Samples of about 1 g of feces which had been stored at -70°C were emulsified in 10 mL of PBS and filtered through Swinnex (Millipore Ltd., Mississauga, Ontario) filters with a glass fibre prefilter, then 1.2 μ and 0.45 μ poresizes. Young (6 to 18 h) monolayers of Crandell's feline kidney cell line (CRFK) were inoculated with the fecal filtrates. Cultures with no virus hemagglutinin in supernatants after five days at 37°C were trypsinized and reestablished for a further five days. Isolation of virus on first passage was interpreted as indicative of relatively heavy shedding of virus. Isolation only in trypsinized second passage cultures was considered to indicate shedding of virus in low concentration. Failure to demonstrate hemagglutinin in trypsinized cultures after the five days reincubation was interpreted as no shedding of virus.

Attempts were made to culture from the first feces passed after inoculation (which was usually not until 2 or 3 DAI, due to the fasting period imposed), from feces passed 4 or 5 DAI, and from feces passed during the period 8-10 DAI.

SEROLOGY OF WILD ANIMALS

In addition to samples from foxes and raccoons collected directly from the wild specifically for this study, sera were obtained from blood collected from free-ranging foxes, raccoons, skunks and coyotes trapped during epizootiological studies on rabies. These animals were captured in rural areas in southwestern Ontario

TABLE I. Number of Animals Inoculated (n), Size of Inoculum and Duration of Observations on Mink, Skunk, Red Fox and Raccoon Given Mink Virus Enteritis (MVE), Canine Parvovirus (CPV-2) and Feline Panleukopenia Virus (FPV)

	MVE			CPV			FPV		
	n	Days Observed	Inoculum ($TCID_{50}$)[a]	n	Days Observed	Inoculum ($TCID_{50}$)	n	Days Observed	Inoculum ($TCID_{50}$)
Mink	2	5[k]	$10^{4.9}$	2	5[k]	$10^{2.9}$	2	5[k]	$10^{7.4}$
	4	12	$10^{4.9}$	4	12	$10^{2.9}$	4	20	$10^{3.7}$
Skunk	2	5[k]	$10^{3.9}$	2	5[k]	NT	2	5[k]	$10^{7.4}$
	4	21	$10^{3.9}$	4	21	NT	4	21	$10^{7.4}$
Raccoon	1	5[k]	$10^{4.9}$	1	5[k]	$10^{2.9}$	5	21	$10^{3.7}$
	1	5[k]	$10^{3.9}$						
	4	21	$10^{3.9}$	7	12-21	$10^{2.9}$			
Fox	2	5[k]	$10^{4.9}$	6	15-21	$10^{2.8}$	2	16-21	$10^{7.4}$
	3	12	$10^{4.9}$						

[a]Tissue culture mean infective doses
[k]Animals killed and tissues recovered for microscopic examination
NT = Not titratable

TABLE II. Serological Response (reciprocal HAI titre) and Shedding of Virus by Mink Inoculated with Mink Virus Enteritis (MVE), Feline Panleukopenia Virus (FPV) and Canine Parvovirus (CPV-2)

		Days After Inoculation														
Virus Inoculated	Mink No.	0	1	2	3	4	5	6	7	8	9	10	11	12	16	20
MVE	MM-1[k]	<8~~V~~					V									
	MM-2[k]	<8~~V~~					V									
	MM-3	<8~~V~~					V					V		256		
	MM-4	<8~~V~~					V					V		256		
	MM-5	<8~~V~~					V					~~V~~		768		
	MM-6	<8~~V~~					V					~~V~~		192		
FPV	MF-1[k]	<8		<8~~V~~	~~V~~	24~~V~~	24									
	MF-2[k]	<8		32~~V~~	~~V~~	12~~V~~	<8~~V~~									
	MF-3	<8	V			v	16			256	v			256	128	256
	MF-4	<8	V			V	24			256~~V~~				256	192	192
	MF-5	<8	~~V~~			~~V~~	32			384~~V~~				384	256	192
	MF-6	<8	V			~~V~~	12			384~~V~~				256	192	192
CPV-2	MC-1[k]	<8		<8		<8										
	MC-2[k]	<8		<8		<8										
	MC-3	<8		<8		<8v		8		192~~V~~				>512		
	MC-4	<8~~V~~		<8		<8~~V~~		12		256v				>512		
	MC-5	<8~~V~~		<8		<8~~V~~		8		256~~V~~				>512		
	MC-6	<8~~V~~		<8		<8~~V~~		<8		192~~V~~				>512		

[k]Killed 5 DAI; ~~V~~ — no virus isolated from feces; V — virus isolated 1st passage; v — virus isolated 2nd passage

near Lake Huron. Sera were frozen at -20°C until used. Titrations were performed by HAI as described above.

RESULTS

Serological response to inoculation of virus, and shedding of virus by the four species is summarized in Tables II-V. These results, together with the pathological and clinical response by each species to the inoculations are described below.

MINK

Mink inoculated with MVE all showed signs of infection for variable periods between 3 and 12 DAI. These included inappetance, mucoid grey, or bile- or blood-tinged diarrhea, and depression indicated by lassitude and inactivity. Animals remained curled in a corner of the cage, unresponsive to the activities of the animal attendant. No animals died and all but one, which was not eating, seemed normal by 12 DAI. Lesions typical of early parvovirus infection were present in the two animals killed 5 DAI. In some parts of the small intestine of one animal dilated crypts of Lieberkuhn were lined by attenuated epithelial cells with large nuclei and prominent nucleoli. Exfoliated epithelium and neutrophils were in the gland lumen. Flattened cells were present on stumpy villi subtended by damaged crypts. In the small intestine of both mink, numerous crypt lining cells were swollen with indistinct edges and contained nuclei with marginated chromatin and prominent intranuclear inclusions. Connective tissue between crypts was condensed and infiltrated by numerous eosinophils. Glands lining the fundic stomach in the more severely affected mink had attenuated neck cells lining the isthmus of the gland, and dilatation of the neck of the gland in some cases (Fig. 1). Gut-associated lymphoid tissue was moderately involuted but not necrotic. Some splenic follicles were hyalinized; many had peripheral populations of proliferating lymphocytes, with occasional foci of lymphocytolysis and rare cells containing large intranuclear inclusions.

All mink surviving to 12 DAI had high titres to parvovirus antigen, and virus was isolated at first passage from all animals 5 DAI and from two of four mink 10 DAI (Table II).

Inoculation of FPV caused mild signs of illness in mink. Reduction in or loss of appetite, accompanied by sporadic mucoid diarrhea and occasional depressed demeanor was present for varying periods during the interval 3-12 DAI.

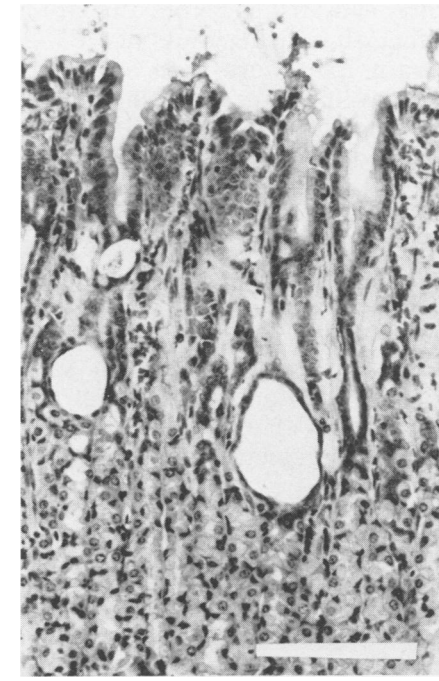

Fig. 1. Attenuation of epithelium lining the isthmus of gastric fundic glands, associated with narrowing or dilatation of the gland in a mink five days after inoculation with mink virus enteritis. Scale bar = 100 μm H & E.

Signs were not as severe as in mink with MVE, and were most consistent in the two animals which had virus in feces 4 and/or 9 DAI (Table II). Virus was not isolated from the other animals except on the day after inoculation. Typical intranuclear inclusions were present in some crypt epithelial cells

TABLE III. Serological Response (reciprocal HAI titre) and Shedding of Virus by Skunks Inoculated with Mink Virus Enteritis (MVE), Feline Panleukopenia Virus (FPV) and Canine Parvovirus (CPV-2)

Virus Inoculated	Skunk No.	Days After Inoculation 0	1	2	3	4	5	6	7	8	12	16	21
MVE	SM-1[k]	<8		12 V		<8	<8V						
	SM-2[k]	<8		12 V		<8	<8V						
	SM-3	<8		16	V	8	<8V			12V	<8	12	12
	SM-4	<8		16	V	8	<8V			12V	16	16	8
	SM-5	<8		12	V	<8	<8V			24V	12	<8	12
	SM-6	<8		8	V	<8	<8V			24V	12	12	12
FPV	SF-1[k]	<8		<8 V		12V	24						
	SF-2[k]	<8		<8	V	32V	12						
	SF-3	<8		12 V		12	32	V		16V	<8	32	48
	SF-4	<8		<8	V	32	12			12V	8	12	<8
	SF-5	<8		<8	V	24	16V			48V	8	16	24
	SF-6	<8		16	V	32	24V			16V	24	24	16
CPV-2	SC-1[k]	<8		<8		12	<8V						
	SC-2[k]	<8		24 V		<8	<8V						
	SC-3	<8		12 V		16	8V			<8V	24	24	8
	SC-4	<8		12	V	<8	V			<8V	12	16	8
	SC-5	<8		<8V		8	<8V			8V	32	16	24
	SC-6	<8		16 V		<8	<8V			8V	32	16	16

[k]Killed 5 DAI; V — no virus isolated from feces

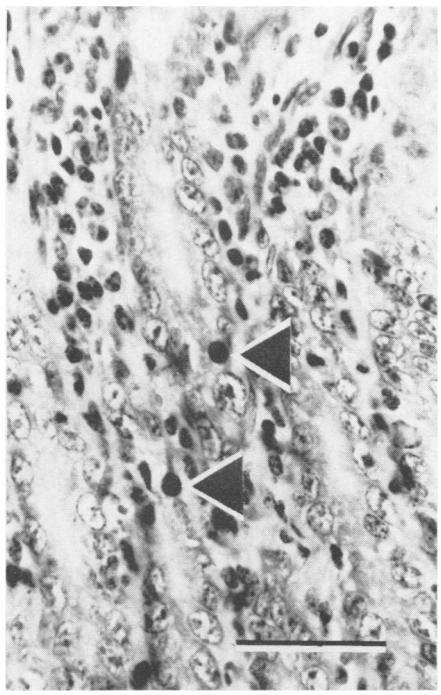

Fig. 2. Epithelial cells in the crypts of Lieberkuhn of the small intestine, with open nuclei, prominent nucleoli, and some condensed nuclei containing basophilic inclusions (arrows). Mink five days after inoculation with feline panleukopenia virus. Scale bar = 30 μm H & E.

(Fig. 2) but no other microscopic lesions were evident in the tissues of the two mink killed 5 DAI. A moderately strong serological response to parvovirus antigen was elicited in the four animals followed until 20 DAI (Table II).

Canine parvovirus-2 inoculation was associated with mild signs of inappetance (about half the normal ration eaten 4-6 DAI) in three of four mink followed to 12 DAI. No lesions were evident in the tissues of one of the two animals killed 5 DAI; however in the second animal crypt epithelial cells, some with equivocal inclusions, were exfoliating (Fig. 3), though crypts were not dilated. A strong serological response to inoculation was evident 12 DAI (Table II). Virus was isolated on second passage from the feces of two of four animals, one at each of 4 and 8 DAI (Table II).

SKUNK

Skunks appeared to be refractory to infection with all three parvoviruses. No signs of illness were noted; no lesions were found in animals killed 5 DAI; and virus was not isolated from the feces (Table III). Seroconversion, when it did occur, was at very low titre and inconsistently detectable (Table III).

RED FOX

Foxes showed little or no response to inoculation with MVE. None of the three animals followed to 12 DAI showed signs of illness, only one of the three seroconverted to low titre (Table IV) and no lesions or viral inclusions were evident in the two animals killed 5 DAI. However, virus was recovered on second passage from the feces of four of five animals 5 or 10 DAI (Table IV).

The two foxes inoculated with FPV showed no signs of illness but

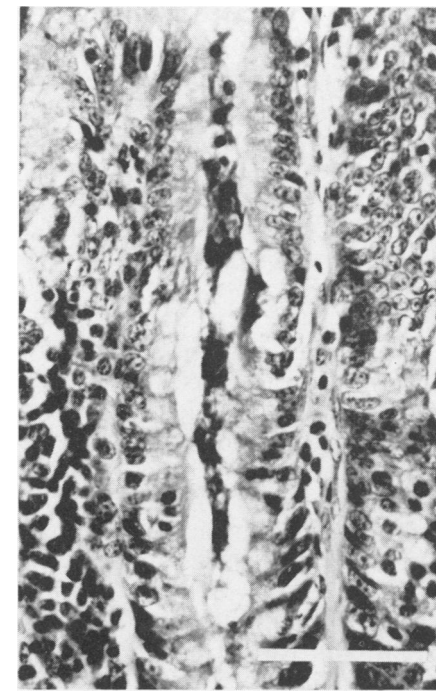

Fig. 3. Crypt of Lieberkuhn containing exfoliated cells and mucus, in small intestine of a mink inoculated with canine parvovirus-2 and killed five days after inoculation. Scale bar = 50 μm H & E.

strong serological responses to inoculation (Table IV). Virus was isolated on second passage from one of these animals 2-4 DAI but shedding of virus was not persistent (Table IV). Insufficient experimental animals were available to permit killing at 5 DAI to examine for lesions.

Canine parvovirus-2 inoculation elicited no signs of illness in six foxes monitored for 15-21 days. All seroconverted weakly, with a max-

TABLE IV. Serological Response (reciprocal HAI titre) and Shedding of Virus by Red Foxes Inoculated with Mink Virus Enteritis (MVE), Feline Panleukopenia Virus (FPV) and Canine Parvovirus (CPV-2)

Virus Inoculated	Fox No.	Days After Inoculation																	
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	21
MVE	FM-1[k]	<8	V				v												
	FM-2[k]	<8Ψ	v																
	FM-3	<8Ψ					v					v		64					
	FM-4	<8Ψ					Ψ					v		<8					
	FM-5	<8Ψ					v					Ψ		<8					
FPV	FF-1	<8	Ψ	<8v	v	<8v	Ψ	Ψ	Ψ	384Ψ	Ψ	Ψ		1024				768	
	FF-2	<8		<8Ψ	Ψ	32Ψ	Ψ	Ψ	Ψ	32Ψ	Ψ	Ψ		256				512	256
CPV-2	FC-1	<8		V	V	Ψ	Ψ	Ψ	Ψ	v				<8		16			16
	FC-2	<8	Ψ			Ψ	<8			24Ψ				24			32		
	FC-3	<8	Ψ			Ψ	<8			16Ψ				16			<8		
	FC-4	<8	Ψ			Ψ	<8			32Ψ				32			<8		
	FC-5	<8		v		Ψ	<8			24Ψ				24			12		
	FC-6	<8		v			<8Ψ			64Ψ				64			8		

[k]Killed 5 DAI; Ψ — no virus isolated from feces; V — virus isolated 1st passage; v — virus isolated 2nd passage

imum titre of 1:64 (Table IV). Virus which may have been from the inoculum was isolated from the feces of three of six foxes 2-3 DAI, but virus was detected, on second passage, from the feces of only one animal later in the experiment (Table IV). No animals were killed for examination of lesions.

RACCOON

Mink virus enteritis was highly virulent for raccoons. One of two animals killed 5 DAI, and all four raccoons observed for longer periods showed signs of illness during the period 4-12 DAI. They lost their appetite, became profoundly depressed, and curled in the back of their cages. All developed mucoid or foul watery diarrhea which was tinged with blood or overtly bloody.

Illness culminated in the death of two animals 8 and 9 DAI. At necropsy they had severe hyperemia of the mucosa of the large and small intestine, hemorrhagic Peyer's patches and flecks of fibrin adherent to the mucosa of the gut. Mesenteric lymph nodes were edematous and enlarged. Petechial hemorrhages were present in the thymus. Microscopic examination revealed severe necrosis of crypt epithelium, loss of crypts, collapse of villi and erosion of the mucosa throughout the intestine (Fig. 4). There was severe necrosis and involution of lymphoid tissue in Peyer's patches, mesenteric lymph node, thymic cortex and tonsil. Similar microscopic lesions were present in one of the two animals killed 5 DAI, which had shown signs of depression and loss of appetite for one day. The other raccoon killed 5 DAI was very young, did not show signs, but had early lesions with exfoliation of cryptal epithelium in the small intestine and colon, and prominent intranuclear inclusions in megakaryotic crypt epithelial cells (Fig. 5). Necrosis and involution of gut-associated lymphoid tissue was also evident. Other lymphoid elements throughout the body

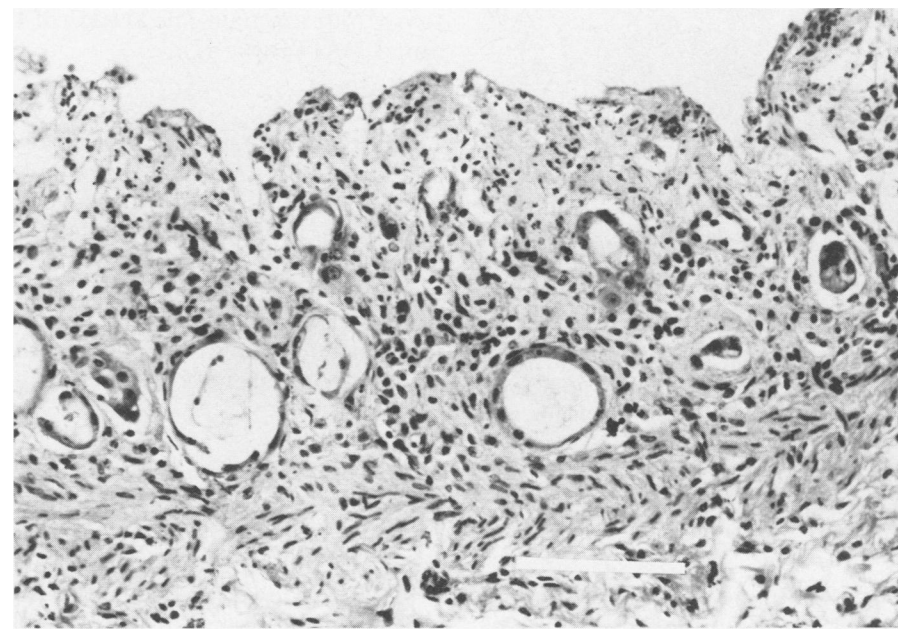

Fig. 4. Severe damage to crypts of Lieberkuhn, collapse of villi and erosion of mucosal surface in small intestine of a raccoon dying eight days after inoculation with mink virus enteritis. Scale bar = 50 μm H & E.

TABLE V. Serological Response (reciprocal HAI titre) and Shedding of Virus by Raccoons Inoculated with Mink Virus Enteritis (MVE), Feline Panleukopenia Virus (FPV) and Canine Parvovirus (CPV-2)

Virus Inoculated	Raccoon No.	Days After Inoculation 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	20	21
MVE	RM-1[k]	<8 Ψ	Ψ		v	V	192 Ψ													
	RM-2[k]	<8	Ψ				96 V													
	RM-3	<8								512	V			384				512		512
	RM-4	<8 Ψ					24	Ψ		256 V				384				2048		1536
	RM-5[D]	<8		V		<8	V		V	512										
	RM-6[D]	<8		Ψ		<8 V			V	768										
FPV	RF-1	<8			V	<8 V				512 V				512					384	
	RF-2	<8			v	<8 V				256 V				384					768	
	RF-3[D]	<8			v	<8 V		128												
	RF-4[D]	<8	v			<8 V				256 V		256								
	RF-5	<8			v	<8 V				256 V				512					192	
CPV-2	RC-1[k]	<8				<8	12													
	RC-2	<8 Ψ			Ψ		<8			96 v				128			256			
	RC-3	<8			Ψ	Ψ	<8			96 v				192				256	192	
	RC-4	<8			Ψ	Ψ	<8			256	Ψ			512				512	256	
	RC-5	<8			Ψ	Ψ	<8			192 Ψ				384				384	768	
	RC-6	<8 Ψ		<8		<8 Ψ		<8		8 Ψ		<8								
	RC-7	<8			Ψ	Ψ	Ψ		Ψ				Ψ	<8		12				24
	RC-8	<8			v	Ψ	Ψ		Ψ				Ψ	<8						<8

[k]Killed 5 DAI; [D]died of parvovirus disease; Ψ — no virus isolated from feces; V — virus isolated 1st passage; v — virus isolated 2nd passage

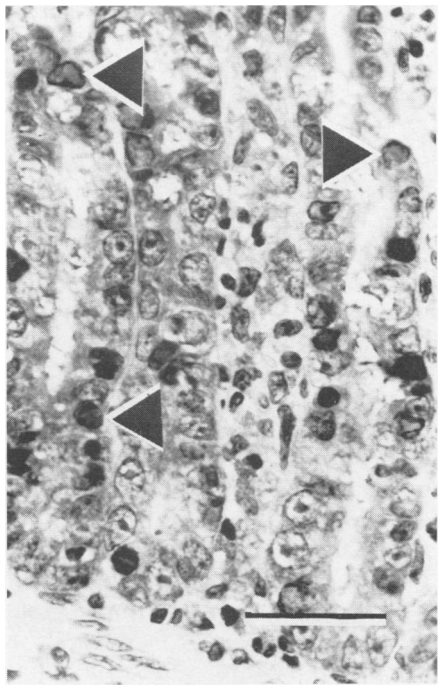

Fig. 5. Epithelium in the crypts of Lieberkuhn in the small intestine, containing large open nuclei with prominent nucleoli, or basophilic intranuclear inclusions (arrows). Raccoon, five days after inoculation with mink virus enteritis. Scale bar = 30 μm H & E.

appeared normal in this animal.

Seroconversion to high titre followed MVE inoculation in raccoons (Table V) and virus was isolated on first passage from the feces of all animals during the period 4-9 DAI (Table V). One animal which died had a direct virus hemagglutinin titre in feces exceeding 3.2×10^7, the day before it died.

Feline panleukopenia was similarly highly virulent for raccoons. All five animals inoculated showed signs of infection over the period 3-11 DAI. These varied from some looseness of stool to mucoid or watery diarrhea with blood or fibrin, loss of appetite, and severe depression as was seen with MVE infection. Two animals died, 6 and 10 DAI. Gross and microscopic lesions in the animals which died resembled those described above in animals dead with MVE, except that involution of splenic lymphoid elements occurred, there was severe attenuation of epithelium in the upper portions of gastric glands, and there was severe pulmonary congestion and edema. Hemolytic *Escherichia coli* were septicemic in both animals. All five raccoons responded strongly serologically to FPV (Table V) and virus was isolated at first passage from the feces of each animal 4 and 8 DAI, indicating heavy and persistent shedding of virus.

Response by raccoons to CPV-2 was mild. Signs of illness were not seen in seven animals followed for 12-21 DAI, nor were lesions or unequivocal intranuclear inclusions evident in the single animal killed 5 DAI. Two animals failed to respond serologically to inoculation, one did so weakly, but four others developed moderately high titres of antibody (Table V). Virus was detected on second passage in the feces of only two animals, once at 8 DAI and once at 3 DAI. In the latter case virus was in the first feces passed after the fasting period and may represent virus from the inoculum.

PREVALENCE OF ANTIBODY TO PARVOVIRUS IN WILD CARNIVORES

The prevalence of antibody, and the titre ranges, found in 143 wild red fox, 112 raccoon and 157 skunk trapped or captured at several localities in southern Ontario during the fall of 1980 and 1981 are summarized in Fig. 6. The prevalence of antibody (titres $\geq$1:8) was highest in foxes (114/144, 79.2%), contrasted with that in raccoons (25/112, 22.3%) and skunks (2/157, 1.3%). There were no differences in either the occurrence of antibody or the level of titre in male and female foxes. However there were significantly more foxes with antibody in 1981 than in 1980. The number of raccoons and skunks with antibody was greater in 1981 also but differences were not significant. In both years the percentages of juveniles and adults sampled was the same. The percentage of samples with high titres >1:256) was 12.5% in raccoons and 10% in foxes. No skunks had titres greater than 1:192. In addition seven coyote sera were tested and six had antibody (titre range 1:192 to 1:1536).

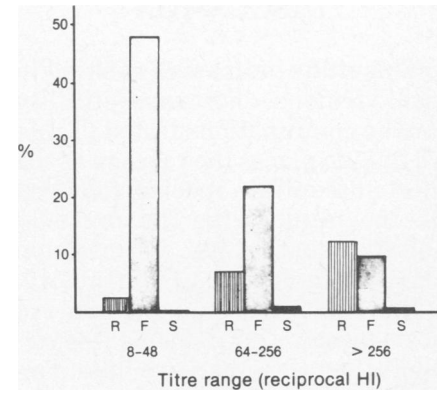

Fig. 6. Prevalence of hemagglutination-inhibiting antibody to canine parvovirus-2 in raccoons (n = 112), foxes (n = 144) or skunks (n = 157) trapped or captured in southern Ontario 1980/1. Bars represent the percentage of all serum samples from each species with antibody titres in the range indicated; R = raccoon, F = fox, S = skunk.

TABLE VI. Comparison of Severity of Clinical Signs, Degree of Shedding of Virus and Serological Response, among Mink, Striped Skunk, Red Fox and Raccoon Inoculated with Mink Virus Enteritis (MVE), Feline Panleukopenia Virus (FPV) and Canine Parvovirus (CPV-2)

	Signs			Shedding of Virus			Serological Response		
Species	MVE	FPV	CPV-2	MVE	FPV	CPV-2	MVE	FPV	CPV-2
Raccoon	+++	+++	-	+++	+++	+	+++	+++	++
Mink	++	++	+	+++	+	+	+++	++	+++
Fox	-	-	-	+	+	+	+	+++	+
Skunk	-	-	-	-	-	-	+	+	+

Signs: - none; + some diarrhea, inappetance or depression; ++ consistent diarrhea, depression; +++ consistent signs and deaths
Shedding of virus: - none after 3 DAI; + sporadic/2nd passage 4 DAI or later; ++ all animals, some 1st passage 4 DAI or later; +++ all animals, 1st passage 4 DAI or later
Seroconversion: - none; + no animals with titre >64, ++ no animals >384, or highly variable; +++ some or all animals >384

DISCUSSION

This study indicates a ranking in viral virulence/ host susceptibility for the combinations tested (Table VI) which places the raccoon as the most susceptible species, followed by the mink, with the fox only slightly susceptible to infection and the skunk virtually refractory. Of the three viruses, in the range of hosts tested, CPV-2 seems less virulent than the other two members of the group, with slight or nonexistent shedding of virus and no disease, though it did stimulate good serological responses in raccoon and mink. Mink virus enteritis and FPV seem to resemble each other more in terms of host range and virulence than either resembles CPV-2. Feline panleukopenia appears to be slightly less well adapted to mink than MVE, with less shedding of virus, no lesions other than viral inclusions 5 DAI, and no mortality using a dose of virus which killed one of two cats. This supports the contention (7) that MVE and FPV in the heterologous host are relatively less virulent. Under circumstances where death was induced lesions were well advanced by 5 DAI. Milder clinical disease was associated with mild lesions or inclusions only in crypt epithelium at that time.

Variations in response by hosts to the various viruses are unlikely to be due to differences in batch of inoculum, since inocula eliciting weak responses in one species typically produced a strong response in another species, e.g. MVE in skunk and raccoons, or fox and mink (Tables I and VI). Inocula were tested for virulence in the homologous species, either within the experiment (MVE in mink) or without (FPV in cats, CPV-2 in dogs). The CPV-2 inoculum was not lethal to dogs, in which species it is difficult experimentally to induce CPV-2 disease consistently (34).

Ranched mink are routinely vaccinated against MVE which is a significant disease in domestic mink. We are not aware that it is recognized as a disease of wild mink. The skunks, like the mink members of the Mustelidae, appeared naturally resistant to MVE, but we cannot be certain that this lack of response by skunks was not a function of previous exposure to parvoviruses. However there were very few seropositive skunk in the wild (Fig. 6). These findings suggest that broad generalizations about the susceptibility to parvoviruses of carnivores based on their taxonomic relationships (4, 33) may be difficult to make. Another mustelid, the ferret *Putorius putorius* is considered refractory to feline panleukopenia (35) though the neonatal ferret is susceptible to FPV-induced cerebellar hypoplasia. Adult ferrets develop significant antibody titres, but not disease when inoculated with FPV (36). The low virulence of CPV-2 in mink indicates that there is little risk of mink contracting parvoviral enteritis from dogs held on mink ranches.

Our findings confirm the susceptibility of raccoons to FPV (32) and MVE (Alberts *et al*, op. cit.). However, the results of our CPV-2 inoculation in raccoons are difficult to reconcile with the isolation of a CPV-like virus from raccoons dying of parvoviral enteritis (33). That is the only report of the isolation of a parvovirus from raccoons. Virus enteritis compatible with "panleukopenia" has been commonly diagnosed on the basis of microscopic lesions in raccoons at the Ontario Veterinary College over the past 20 years, but we have been unsuccessful in attempts to isolate virus to date. The disease in wild raccoons certainly predates the emergence of CPV-2 in 1978.

In Ontario raccoons are found in rural and urban areas. They often forage near houses, sheds and barns even in developed areas where contact with dogs and cats is likely. Contact with domestic cats makes transmission of FPV to raccoons highly probable. The disease is common in raccoons which have been held in animal shelters and sanctuaries, and as pets, where contact with cats or exposure to FPV is likely. It is not known if MVE circulates among wild raccoons and the possibility that a unique "raccoon" parvovirus exists cannot be dismissed. The significance of parvoviral enteritis to wild populations of raccoons is unclear. Animals certainly are exposed to parvoviruses in the wild (Fig. 6). Based on the observed depressed behaviour of experimental animals, wild raccoons becoming ill might be expected to retire to their dens to die or recover. Most of them probably would not come to human attention, in contrast to the situation with rabies and distemper, where abnormal behaviour makes many affected animals obvious.

The insusceptibility of red fox to infection with CPV-2 (Table IV) was somewhat surprising in light of reports of spontaneous CPV-2 infection in canids other than dogs (21, 22, 23). The strong serological response of fox to inoculation with FPV, and close association of foxes with the urban and rural human environment with its cat population may explain the high prevalence of wild foxes seropositive to parvovirus (Fig. 6). The large numbers of seropositive animals encountered during 1981, many of which were young pups with low levels of possibly maternal antibody, confounded attempts to carry out more inoculations of FPV in foxes. Observations on this interaction should be extended, though on the basis of present information it seems unlikely that disease will occur experimentally or in nature due to canine parvovirus. An antigenically related parvovirus specific to the fox should be considered also.

The four species for which sera from wild-ranging individuals were available showed differences in prevalence. These differences may be due to different antibodies, species-specific response rates or disease contact rates. All individuals were trapped in the same rural areas but the movements and activity of these species are different. Coyotes and foxes travel most extensively and skunks the least. Coyotes and foxes showed the highest response rate and skunks the least. However, all four species

but especially skunks and raccoons do come into close contact with man or his domestic pets and all four species are known to ingest feces of other carnivores. Thus, contact rates to parvovirus should not be greatly different among these species. Antibody differences or species-specific susceptibility are a more likely explanation.

Raccoons proved insusceptible to CPV-2 and virus isolated from raccoons appeared more closely related to FPV than CPV-2 (37).

ACKNOWLEDGMENTS

This work was supported by a grant from the Ontario Ministry of Natural Resources, and in part by the Ontario Ministry of Agriculture and Food. We wish to thank Gareth Jones, Zbigniew Wojcinski, Robert Tremblay, Margaret Montgomery, Elmer Ewert and Dr. Susy Carman for their assistance. We gratefully acknowledge the efforts of I. Watt, F. Matecjka, W. Lintack, R. Bramwell and P. Bachmann of the O.M.N.R. Rabies Research Unit in obtaining animals and serum samples. Dr. K. Lawson, Connaught Laboratories, kindly supplied the MVE challenge material.

REFERENCES

1. **HAMMON WD, ENDERS JF.** A virus disease of cats, principally characterized by aleukocytosis, enteric lesions, and presence of intranuclear inclusion bodies. J Exp Med 1939; 69:327-352.
2. **GOSS LJ.** Species susceptibility to the viruses of Carre and feline enteritis. Am J Vet Res 1948; 9:65-68.
3. **BITTLE JL.** Feline panleukopenia. In: Davis JR, Karstad LH, Trainer, D, eds. Infectious diseases of wild mammals. Ames: Iowa State Press, 1970: 85-89.
4. **FOWLER ME, THEOBALD J.** Immunizing procedures. In: Fowler ME, ed. Zoo and wild animal medicine. Philadelphia, Toronto: W.B. Saunders, 1978: 613-617.
5. **JOHNSON RH.** Isolation of a virus from a condition simulating feline panleucopaenia in a leopard. Vet Rec 1964; 76:1008-1012.
6. **JOHNSON RH, SIEGL G, GAUTSCHI M.** Characteristics of feline panleucopenia virus strains enabling definitive classification as parvoviruses. Arch ges Virusforsch 1974; 46:315-324.
7. **BURGER D, GORHAM JR.** Mink virus enteritis. In: Davis JR, Karstad LH, Trainer D, eds. Infectious diseases of wild mammals. Ames: Iowa State Press, 1970: 76-84.
8. **WILLS CG.** Notes on infectious enteritis of mink and its relationship to feline enteritis. Can J Comp Med 1952; 16:419-420.
9. **KAARIAINEN L, KANGAS T, KERANEN S, NYHOLM M, WECKSTROM P.** Studies on mink enteritis virus. Arch ges Virusforsch 1966; 14:197-209.
10. **JOHNSON RH.** Feline panleucopaenia virus - *in vitro* comparison of strains with a mink enteritis virus. J Small Anim Pract 1967; 8:319-323.
11. **MacPHERSON LW.** Feline enteritis virus. Its transmission to mink under natural and experimental conditions. Can J Comp Med 1956; 20:197- 202.
12. **BURGER D.** The relationship of mink virus enteritis to feline panleukopenia virus. M.S. Thesis, Washington State University, Pullman (cited by Burger and Gorham 1970).
13. **MYERS WL, ALBERTS JO, BRANDLY CA.** Certain characteristics of the virus of infectious enteritis of mink and observations on the pathogenesis of the disease. Preliminary report. Can J Comp Med 1959; 23:282-287.
14. **EUGSTER AK, BENDELE RA, JONES LP.** Parvovirus infection in dogs. J Am Vet Med Assoc 1978; 173:1340-1341.
15. **KELLY WR.** An enteric disease of dogs resembling feline panleucopaenia. Aust Vet J 1978; 54:593.
16. **THOMSON GW, GAGNON AN.** Canine gastroenteritis associated with a parvovirus-like agent. Can Vet J 1978; 19:346.
17. **APPEL MJG, SCOTT FW, CARMICHAEL LE.** Isolation and immunization studies of a canine parvo-like virus from dogs with haemorrhagic enteritis. Vet Rec 1979; 105:156-159.
18. **GAGNON A, POVEY RC.** A possible parvovirus associated with an epidemic of gastroenteritis in dogs in Canada. Vet Rec 1979; 104:263-264.
19. **JOHNSON RH, SPRADBROW PB.** Isolation from dogs with severe enteritis of a parvovirus related to feline panleucopaenia virus. Aust Vet J 1979; 55:151.
20. **BINN LN, LAZAR EC, EDDY GA, KAJIMA M.** Recovery and characterization of a minute virus of canines. Infect Immun 1970; 1:503-508.
21. **FLETCHER KC, EUGSTER AK, SCHMIDT RE, HUBBARD GB.** Parvovirus infection in maned wolves. J Am Vet Med Assoc 1979; 175: 897-900.
22. **MANN PC, BUSH M, APPEL MJG, BEEHLER BA, MONTALI RJ.** Canine parvovirus infection in South American canids. J Am Vet Med Assoc 1980; 177:779-783.
23. **EVERMANN JF, FOREYT W, MAAG-MILLER L, LEATHERS CW, McKEIRNAN AJ, LEAMASTER B.** Acute haemorrhagic enteritis associated with canine coronavirus and parvovirus infections in a captive coyote population. J Am Vet Med Assoc 1980; 177:784-786.
24. **CARMICHAEL LE, JOUBERT JC, POLLOCK RVH.** Haemagglutination by canine parvovirus: serologic studies and diagnostic applications. Am J Vet Res 1980; 41:784-791.
25. **LENGHAUS C, STUDDERT MJ.** Relationships of canine panleucopaenia (enteritis) and myocarditis parvoviruses to feline panleucopaenia virus. Aust Vet J 1980; 56:152-153.
26. **FLOWER RLP, WILCOX GE, ROBINSON WF.** Antigenic differences between canine parvovirus and feline panleucopenia virus. Vet Rec 1980; 107:254-256.
27. **McMASTER GK, TRATSCHIN J-D, SIEGL G.** Comparison of canine parvovirus with mink enteritis virus by restriction site mapping. J Virol 1981; 38:368-371.
28. **TRATSCHIN J-D, McMASTER G, HIRT B, SIEGL G.** Comparison of canine parvovirus with feline panleucopenia virus and mink enteritis virus. Abstracts Fifth Int Cong Virol Strasbourg 1981:369.
29. **PHILLIPS CE.** Haemorrhagic enteritis in the artic (sic) blue fox. Caused by the virus of feline enteritis. Can J Comp Med 1943; 7:33-35.
30. **NAIR KPD, PADMANABHA IYER R, VENUGOPALAN A.** An outbreak of gastroenteritis in civet cats. Indian Vet J 1964; 41:763-765.
31. **JOHNSON RH, HALLIWELL REW.** Natural susceptibility to feline panleucopaenia of the coatimundi. Vet Rec 1968; 82:582.
32. **WALLER EF.** Infectious gastroenteritis in raccoons (*Procyon lotor*). J Am Vet Med Assoc 1940; 96:266-268.
33. **NETTLES VF, PEARSON JE, GUSTAFSON GA, BLUE JL.** Parvovirus infection in translocated raccoons. J Am Vet Med Assoc 1980; 177:787-789.
34. **CARMAN S, POVEY C.** Successful experimental challenge of dogs with canine parvovirus-2. Can J Comp Med 1982; 46:33-38.
35. **RYLAND LM, GORHAM JR.** The ferret and its diseases. J Am Vet Med Assoc 1978; 173:1154-1158.
36. **JOHNSON RH, MARGOLIS G, KILHAM L.** Identity of feline ataxia virus with panleucopenia virus. Nature 1967; 214:175-177.
37. **APPEL MJG, PARRISH CR.** Raccoons are not susceptible to canine parvovirus. J Am Vet Med Assoc 1982; 181:489.