

ROTAVIRUS-ASSOCIATED DIARRHEA IN YOUNG RACCOONS (PROCYON LOTOR), STRIPED SKUNKS (MEPHITIS MEPHITIS) AND RED FOXES (VULPES VULPES)

Author: Evans, R. H.

Source: Journal of Wildlife Diseases, 20(2) : 79-85

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-20.2.79>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

ROTAVIRUS-ASSOCIATED DIARRHEA IN YOUNG RACCOONS (*PROCYON LOTOR*), STRIPED SKUNKS (*MEPHITIS MEPHITIS*) AND RED FOXES (*VULPES VULPES*)

R. H. Evans

Treehouse Wildlife Center, Inc., RR 1, Box 125E, Brighton, Illinois 62012, USA

ABSTRACT: Electron microscopy and a commercial ELISA test for rotavirus antigen were used to diagnose rotavirus infection in diarrheic raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*) and red foxes (*Vulpes vulpes*). Gross and histopathological changes in two raccoons and two red foxes were found to be very similar to those described previously in rotavirus mediated diarrhea in other animals. While an etiology for the diarrhea is not definitively established, it would appear to involve rotavirus alone or possibly in concert with enteropathogenic coliform bacteria, overfeeding of a commercial kitten milk replacer and the stresses of captivity.

INTRODUCTION

Rotavirus infections have been associated with diarrhea in a wide variety of neonatal, adolescent and adult domestic and wild animals as well as man. (McNulty, 1978; Woode and Crouch, 1978). This virus is considered one of the most important etiologic agents of neonatal diarrhea in calves, pigs and lambs, frequently resulting in significant economic loss (House, 1978). Rotavirus diarrhea is the most frequent cause of infantile gastroenteritis in human neonates and is an important cause of diarrhea in young children 1-3 yr of age (Banatvala and Chrystie, 1978). A recent survey of captive wild mammals (Petrie et al., 1981) revealed a widespread prevalence of rotaviral antibodies substantiating the ubiquitous nature of this virus in higher mammals. In the order Carnivora, rotavirus antibodies were found in exotic cats, bears, a raccoon dog (*Nyctereutes procyonoides*), coyote (*Canis latrans*), and Arctic wolf (*Canis lupus*) (Petrie et al., 1981). The author recently reported on an outbreak of infectious diarrhea in mink associated with rotavirus (Evans, R. H. Rotavirus-associated diarrhea in mink. Report given before First International Symposium on Viral

Diseases of Mink, National Institute of Health, Rocky Mountain Laboratory for Slow Virus Diseases, Hamilton, Montana February 21-23, 1983). In a study on domestic animals in Ireland (McNulty et al., 1978), 79% of the dogs and 83% of the cats examined had rotaviral antibodies, while other authors found antibodies in 58.5% of 106 dogs examined in Louisiana (Pearson et al., 1980). Rotavirus has been implicated as an etiologic agent of spontaneous diarrhea in young dogs (Eugster and Sidwa, 1979; England and Poston, 1980; Everman et al., 1982; Fulton et al., 1982). Most recently, researchers in Oklahoma have succeeded in experimentally producing rotavirus diarrhea in gnotobiotic dogs following inoculations with an isolate obtained from a pup with fatal diarrhea (Johnson et al., 1983). The following report describes the clinical and pathological findings of rotavirus-associated diarrhea in the young of three species of wild carnivores.

MATERIALS AND METHODS

The raccoons and skunks were admitted to Treehouse Wildlife Center as orphans between January 1979 and May 1983. Each animal was given a thorough physical examination and found to be in good health. Age was determined according to dentition (Montgomery, 1964; Verts, 1967). Following physical examination, the kits were given one dose of a balanced electrolyte replacement fluid in D5W

Received for publication 6 September 1983.



TABLE 1. Clinical course of rotavirus infections in raccoons, striped skunks and foxes.

Species	Age (days)	Onset of diarrhea ^a	Duration of diarrhea
Raccoon	17	21	2
	18		11 ^b
	19		7
Raccoon	38	30-40	3
	39		6
	40		8
	41		8
Skunk	7	18-21	3
	8		7
	9		8
Skunk	19	42-50	2
	20		9
	21		8
Fox ^d	32	56-60	2
	61		Unknown
		Unknown	Unknown

^a Days post admission.^b Animal died on day 11 of diarrhea.^c Animal died on day 13 of diarrhea.^d Animals submitted DOA.

(NormSol-R in Dextrose 5% in water, Ceva Labs, Overland Park, Kansas 66212, USA) at 50 ml/kg of body weight by gavage and then housed in 1-m³ cages in separate wards. Four hr later, feeding with a commercial kitten milk replacer (KMR, Borden, Inc., Norfolk, Virginia 23501, USA) ad libitum three to four times daily was begun. The red foxes were admitted dead-on-arrival (DOA) after being struck by automobiles and were submitted immediately for necropsy. They were aged according to dentition (Linhart, 1968).

Feces were collected from raccoons and skunks at the onset of diarrhea, at irregular intervals during its course, 2-3 days after full recovery was evident and at necropsy from Raccoons 17 and 19 and the two red fox pups. Portions of the feces (fresh or stored less than 72 hr at 4 C) were submitted for negative-contrast electron microscopy (NCEM) (School of Medicine, Department of Pathology, St. Louis University, St. Louis, Missouri 63104, USA), aerobic and anaerobic bacteriology (Veterinary Services Department, Ralston Purina Company, St. Louis, Missouri 63164, USA) and parvovirus isolation attempts on Raccoons 38-41 (James A. Baker Institute of Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA).

For electron microscopy, a small amount of

feces was diluted with sufficient distilled water to make a slurry and this was vortexed and then centrifuged at 3,000 rpm for 5-10 min or until the solution was cleared. One drop of the supernatant from this solution was inoculated onto two or three 200-mesh collodion copper grids and stained for about 10 sec with 1% phosphotungstate stain at pH 7. Grids were then examined under a JEOL 100 CX electron microscope at 60 kV for presence of negatively stained viral particles at 20,000 \times .

Bacterial culturing was performed by inoculation of feces onto blood agar, CDC anaerobic blood agar, MacConkey agar, S-H agar and selenite broth. Cultures were incubated at 37 C for 24-48 hr, aerobically and anaerobically (Forma anaerobic chamber). Colonies observed after incubation were subcultured on selective and non-selective media. Anaerobes were confirmed by inability to grow aerobically on non-selective media. Final identifications were made utilizing rapid biochemical test strips (API-20E, API-20A, API-staphase, Analytab Products, Plainview, New York 11803, USA), conventional biochemical tests as well as colony morphology and microscopic characteristics.

Virus isolation attempts were performed by inoculating monolayers of Crandell feline kidney (CRFK) cells with filtered 10% suspension of fresh feces as described previously (Appel et al., 1979).

Additional portions of feces were also screened for parasites by fecal flotation and ether-extract sedimentation as well as Rotavirus antigen with a commercial ELISA test kit (Rotazyme[®], Abbott Laboratories, North Chicago, Illinois 60664, USA).

Necropsies on raccoons 17 and 19 as well as the two red foxes were performed within 1 hr of death. Gross observations were noted and representative tissues from all major organ systems were taken for histopathology. They were fixed in 10% neutral buffered formalin and submitted to a local hospital pathology lab for routine processing, sectioning at 6 μ m and staining with hematoxylin and eosin (Department of Pathology, Normandy Osteopathic Hospital, St. Louis, Missouri 63121, USA).

RESULTS

Table 1 annotates the clinical course of rotavirus-associated diarrhea in 16 wild carnivores. Within 96 hr of instituting KMR feeding, all animals had frequent loose, semi-formed stools and moderate flatulence. Twenty-four hr later, severe, fulminating diarrhea characterized by ex-

tremely watery, frequently bile-stained and fetid, feces developed. Most animals had 20–35 daily projectile eliminations, while some appeared to have rectal incontinence with almost constant dribbling bowel movements. Additionally, the kits exhibited varying degrees of dehydration, anorexia, abdominal distention, depression and hypothermia. At this time, the kits were taken off KMR, given a balanced electrolyte solution in D5W by gavage at 50 ml/kg two to four times daily and put on a heating pad. A high calorie supplement (Nutrical, EVSCO Pharmaceuticals Corp., Buena, New Jersey 08310, USA) was also added to the fluids daily. Raccoons 19 and 17 were given Amoxicillin (Amoxi-Drops, Beecham Labs, Bristol, Tennessee 37620, USA) at 10 mg/kg twice daily for 7 days.

KMR was reinstituted at 50 ml/kg gradually over a 48 hr period, 7 days after the initiation of fluid therapy. By this time, all but two animals (Raccoons 19 and 17) exhibited either marked improvement or complete recovery. Raccoons 19 and 17 no longer voided watery feces but rather appeared constipated, eliminating every small amounts of tenacious lime-green mucus once or twice daily. Fluid therapy, as outlined above, was alternated with gavage feeding of KMR in Raccoons 19 and 17 until their deaths after 13 and 11 days of diarrhea, respectively. All other animals had completely recovered and were passing well-formed stools at the time these animals died.

At necropsy, gross observations of the raccoons were very similar and included severe emaciation, blanched, thin-walled, moderately fluid-distended jejunum and ileum, small to moderate amounts of straw-colored abdominal fluid, and small amounts of bile-stained mucoid material periodically throughout the colon. The mesenteric lymph nodes were slightly to moderately enlarged and edematous. Examination of the jejunum under the dissecting microscope revealed marked

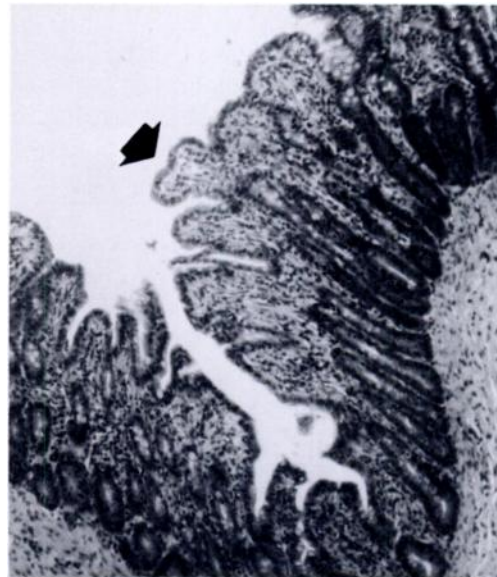


FIGURE 1. Raccoon 19, jejunum. Villi are atrophic and covered with cuboidal, immature absorptive epithelium (arrow). Lateral fusing or cross-bridging is obvious. H&E, 300 \times .

shortening of the villi. Both foxes had suffered massive cranial and thoracic blunt trauma. Their carcasses were emaciated, severely dehydrated and feces pasted their perineums. The jejunum and ileum of both foxes were filled with fetid, brown, frothy fluid and moderate amounts of bile-stained mucus were found throughout the colon.

Varying sized milk curds were noted in the stomachs of both raccoons and foxes. The lungs of the raccoons and foxes were moderately congested and frothy fluid exuded on cut surfaces.

Histologic examination of the jejunum, ileum, colon and associated lymph nodes revealed almost identical lesions in these organs in both the raccoons and foxes. Focal intermittent lesions of the small intestine consisting of varying degrees of degeneration and necrosis of the absorptive epithelium along the distal half of the villi, subsequent contraction of villar lamina propria producing short, blunt villi and lateral villar fusing or epithelial cross-bridging were common (Fig. 1). Many vil-

TABLE 2. Temporal sequence of rotavirus in fecal samples of raccoons, striped skunks and foxes.

Species	Day of diarrhea									
	1	4	5	6	8	10	11	13		
Raccoon	17	ND/ND	+/++	ND/+++	+/ND	ND/ND	ND/+++			
	18	+/+++	ND/ND	ND/ND	-/+	-/-				
	19	+/+++	ND/+++	+/+++	ND/+++	ND/ND	+/+++			
	38	+/+++	+/++	-/+	-/-					
	39	+/+++	+/+++	+/+++	-/+					
	40	+/+++	ND/+++	ND/ND	+/+++	ND/ND	-/-			
	41	+/+++	ND/+++	ND/ND	+/+++	ND/ND	-/-			
Skunk	7	+/+++	+/+++	-/+	ND/ND	ND/ND	-/-			
	8	+/+++	ND/ND	-/-	-/-	-/-				
	9	+/+++	ND/ND	ND/+	ND/+	-/-				
	19	ND/+	+/+++	ND/ND	+/+++	ND/ND	-/+			
	20	+/+++	+/+++	ND/ND	ND/+++	ND/+				
Fox	21	ND/+++	ND/+	ND/ND	-/-					
	32	+/+++	+/+++	+/+++	+/+++					
	61	+/+++	+/+++	+/+++	+/+++					

ND = Not done.

(N) = Sample collected at necropsy.

- Negative contrast electron microscopy—+ = rotavirus present; - = rotavirus absent.

+ Rotazyme® ELISA Test Analysis for rotavirus antigen—- or ± = negative; + = suspect; ++ to ++++ = positive.

li were covered with either squamous or non-vacuolated low cuboidal epithelium. Although true crypt hyperplasia was not seen, crypts were frequently elongated and irregular in outline with markedly increased mitotic activity. The lamina propria of the jejunum, ileum and colon was edematous and contained patchy aggregates of neutrophils, eosinophils and plasma cells. The colon had an irregular or ragged mucosal contour, the result of intermittent patches of low cuboidal epithelium as well as microfoci of surface epithelial necrosis. The crypts and lumen of the colon contained moderate to large amounts of mucus with admixed bacteria and necrotic cellular debris.

Mesenteric and colonic lymph nodes were edematous and exhibited moderate follicular lymphoid hyperplasia with active, sometimes hyalinized germinal centers. Medullary sinuses were filled with numerous neutrophils and plasma cells. Moderate, diffuse interstitial and alveolar edema was found in the lungs of both raccoons and foxes.

A variety of bacterial isolates were cultured from fecal samples including *E. coli* (capsulated, encapsulated, non-hemolytic and hemolytic varieties), *Klebsiella* spp., *Klebsiella oxytoca*, *Proteus* spp. (several biotypes), *Pseudomonas* spp. (several biotypes), *Bacteroides* spp., *Clostridium perfringens* (Raccoons 17 and 41 only), *Streptococcus* spp., *Staphylococcus* spp. and *Bacillus* spp. *Proteus* spp. and/or *E. coli* were consistently isolated from all animals, while other coliforms and *Bacteroides* spp. were the next most common isolates. *Salmonella* spp. were not isolated.

Electron microscopy (EM) and Rotavirus ELISA test (Rotazyme®) results are annotated in Table 2. EM of fecal samples revealed only rotavirus particles whose morphology is illustrated in Figure 2. These particles were consistently present during the period of diarrhea. All fecal examinations for parasites were negative

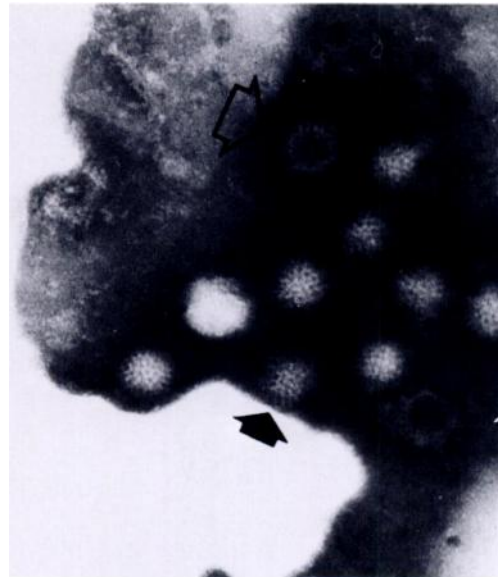


FIGURE 2. NCEM of feces collected 24 hr after onset of diarrhea from Raccoon 39. Rotaviral particles appear as either completely smooth 65 nm particles possessing an outer capsid layer (open arrow) or incomplete particles lacking such a layer (closed arrow). 1% Phosphotungstate stain, 150,000 \times .

in all but Raccoon 18 and the two foxes, where a few unidentified coccidial oocysts were found on two occasions.

Virus isolation attempts were consistently negative after several passages in CRFK cells.

DISCUSSION

The clinical signs, duration of clinical illness, pathological findings and results of diagnostic laboratory tests conducted on the animals in this study are very similar if not identical to those described previously in rotavirus infections in a variety of animals including man (Banatvala, 1978; McNulty, 1978; Woode, 1978). Considering the widespread prevalence of rotaviruses in animals, it is not surprising to find them to be associated with diarrhea in free-ranging and captive non-domestic carnivores.

Despite the large numbers of rotaviral particles seen on EM and the correlation

with Rotazyme® testing, rotaviruses were not isolated from CRFK cells in Raccoons 38–41. This is not surprising, considering the reported difficulty in isolating rotavirus in vitro (McNulty, 1978). In any event, the EM and ELISA results clearly established a rotavirus infection in these animals.

The temporal sequence of positive results of the Negative Contrast Electron Microscopy (NCEM) and Rotazyme® tests correlated very well with the clinical disease. In almost all cases, both tests showed the presence of rotavirus on the first day of diarrhea and continually throughout the course of the clinical disease. Further, the excellent correlation between NCEM and Rotazyme® substantiates previous reports on the suitability of ELISA test kit as a sensitive detector of rotaviral antigen in the feces of dogs, man and other animals (Everman et al., 1982).

Concurrent infections by enteropathogens such as enterotoxigenic *E. coli*, which is a well known synergist in rotaviral diarrhea in cows, pigs and lambs (Woode, 1978) cannot be excluded. *E. coli* were consistently isolated from these animals but their enteropathogenicity was not established. At least it appears likely that secondary bacterial infections contributed in no small part to the demise of Raccoons 17 and 19. This is substantiated by isolating several species of coliforms in very large numbers from the intestines of these animals as well as histological and clinical evidence of inflammatory bowel disease.

Captive management or dietary stresses obviously had no influence on the initiation and course of diarrhea in the fox pups, but such factors must be considered at least as modifying influences in the raccoons and skunks.

It appears that the change from mother's milk to an ad libitum feeding (overfeeding) or KMR substantially contributed to the diarrhea. Rotavirus infections are known to cause a reduction in the brush border enzyme lactase and subsequent

production of osmotic diarrhea. Thus, overfeeding of KMR would be expected to exacerbate rotaviral diarrhea as has been noted in pigs suckling high milk producing sows (Bohl, 1979). We have subsequently determined that feeding KMR at 50 ml/kg body weight per feeding results in a moderately filled stomach and not gastric tympany and/or diarrhea. Conversely, ad libitum feeding will almost routinely result in rapid gastric dilatation and moderate watery diarrhea without pre-existing bowel disease.

Even though definitive proof was lacking, the above data indicated that the diarrhea was caused by either rotavirus alone or in combination with coliform bacteria in the foxes and a combination of rotavirus, overfeeding (as well as dietary change), and possibly enteropathogenic coliforms in the raccoons and skunks.

Alternatively, the cause of diarrhea in the raccoons and skunks could have been the result of overfeeding and dietary change with concurrent coliform infection. This hypothesis relegates the presence of rotavirus to an incidental, subclinical infection. The author feels that this hypothesis is inconsistent with the many previous reports on the role of rotaviruses in suckling mammal diarrhea (McNulty, 1978).

Since the diarrhea in the raccoons and skunks began within 4 days of admission and the reported incubation period in experimentally inoculated dogs can be as short as 24 hr, it is all but impossible to speculate on whether infection occurred prior to or after admission. However, there is no doubt that the foxes contracted their infections in the wild, thus the author feels it is safe to assume that at least some raccoons and skunks were infected prior to admission.

ACKNOWLEDGMENTS

My sincere thanks to Mrs. Terry Melsheimer and to Mr. Jim Gary for their excellent technical assistance during this study and to Mrs.

Cindy Derus for her help in the preparation of this manuscript.

LITERATURE CITED

- APPEL, M. J. G., F. W. SCOTT, AND L. E. CARMICHAEL. 1979. Isolation and immunization studies of a canine parvo-like virus from dogs with haemorrhagic enteritis. *Vet. Rec.* 105: 156-159.
- BANATVALA, J. E., AND I. L. CHRYSTIE. 1978. Rotaviral infections in human neonates. *J. Am. Vet. Med. Assoc.* 173: 527-530.
- BOHL, E. J. 1979. Rotaviral diarrhea in pigs: Brief review. *J. Am. Vet. Med. Assoc.* 173: 613-615.
- ENGLAND, J. J., AND R. P. POSTON. 1980. Electron microscopic identification and subsequent isolation of a rotavirus from a dog with fatal neonatal diarrhea. *Am. J. Vet. Res.* 41: 782-783.
- EUGSTER, A. K., AND T. SIDWA. 1979. Rotavirus in diarrheic feces of a dog. *Vet. Med. Small Anim. Clin.* 74: 817-819.
- EVERMAN, J. F., S. STANN, R. F. DIGIACOMO, P. K. BERGSTROM, A. J. MCKEIRNAN, AND W. E. GIDDENS, JR. 1982. Epizootologic and diagnostic features in canine diarrheal disease in high and low rich dog populations. *Annu. Proc. Am. Assoc. Vet. Lab. Diagnost.* 25: 229-242.
- FULTON, R. W., C. A. JOHNSON, N. J. PEARSON, AND G. N. WOODE. 1981. Isolation of a rotavirus from a newborn dog with diarrhea. *Am. J. Vet. Res.* 42: 841-843.
- HOUSE, J. A. 1978. Economic impact of rotavirus and other neonatal disease agents of animals. *J. Am. Vet. Med. Assoc.* 173: 573-576.
- JOHNSON, C. A., R. W. FULTON, W. G. HENK, AND T. G. SNIDER. 1983. Inoculation of neonatal gnotobiotic dogs with a canine rotavirus. *Am. J. Vet. Res.* 44: 1682-1686.
- , T. G. SNIDER, R. W. FULTON, AND D. CHO. 1983. Gross and light microscopic lesions in neonatal gnotobiotic dogs inoculated with a canine rotavirus. *Am. J. Vet. Res.* 44: 1687-1693.
- LINHART, S. B. 1968. Dentition and pelage in the juvenile red fox (*Vulpes vulpes*). *J. Mammal.* 49: 526-528.
- MCNULTY, M. S. 1978. Rotaviruses. *J. Gen. Virol.* 40: 1-18.
- , G. M. ALLAN, D. J. THOMPSON, AND J. D. O'BROYLE. 1978. Antibody to rotavirus in dogs and cats. *Vet. Rec.* 102: 534.
- MONTGOMERY, G. G. 1964. Tooth eruption in preweaned raccoons. *J. Wildl. Manage.* 28: 582-584.
- PEARSON, N. J., R. W. FULTON, C. A. JOHNSON, G. N. WOODE, AND S. B. BRIETTSCHWERDT. 1980. *Annu. Proc. Am. Assoc. Vet. Lab. Diagnost.* 23: 129-138.
- PETRIE, M., P. J. MIDDLETON, W. A. RAPHEY, K. G. MEHREN, AND C. GRANT. 1981. A survey of zoo mammals for antibody to rotavirus. *Can. J. Comp. Med.* 45: 327-329.
- VERTS, B. J. 1967. *The Biology of the Striped Skunk*. Univ. of Ill. Press, Urbana, Illinois, 218 pp.
- WOODE, G. N., AND C. F. CROUCH. 1978. Naturally occurring and experimentally induced rotaviral infections in domestic and laboratory animals. *J. Am. Vet. Med. Assoc.* 173: 522-526.