Characterization of a New Calicivirus Isolated from 
Feces of a Dog

By

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With 5 Figures

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Summary

Canine calicivirus (CaCV), isolated from feces of a dog with diarrhea, was readily propagated in cultures of canine cells and in a dolphin cell line. Serologic evidence indicated many dogs in at least one geographic area had been infected with CaCV, but its role as an etiologic agent of disease was not established. In cell culture most CaCV virions were strongly cell-associated making purification difficult. CaCV was established as a member of the Caliciviridae by morphology and physicochemical properties of virions (density, sedimentation rate, single major polypeptide, RNA genome size), although some of the properties differed slightly from those of previously described caliciviruses; evidence was also obtained for caliciviral RNA species in infected cells. Based on tests with antisera to numerous caliciviruses and presumed caliciviruses, CaCV appeared to be not closely related to any previously described virus except the stunting syndrome agent of chickens.

Introduction

Particles with calicivirus morphology have been reported to be the putative causal agents of gastroenteritis in humans (7, 9, 10, 18, 21), swine (25), and cattle (3, 40); the Norwalk gastroenteritis agent of humans may
also be a calicivirus (14) although its morphology is not well defined. Despite considerable effort, propagation of these agents in cell culture, an achievement which would permit detailed characterization, has met with little success. Finding a culturable model for extensive study of calicivirus gastroenteritis would be a valuable asset. The recognized members of the family Caliciviridae (27), vesicular exanthema of swine virus (VESV), feline calicivirus (FCV), and San Miguel sea lion virus (SMSV), have been isolated on occasion from the gastrointestinal tract or feces of swine (19), cats (13), and pinnipeds (30), however these viruses are not ordinarily considered to be gastroenteric pathogens. Similarly, a new bovine calicivirus isolated by rectal swab was not associated with gastrointestinal illness (32) and calicivirus-like particles associated with infectious stunting syndrome in chickens were found in feces (42).

This report concerns a virus, isolated from the feces of a diarrheic dog which can be propagated easily in canine cell culture. We initiated investigation of this virus, designated CaCV for canine calicivirus, as a possible model agent for the study of caliciviral gastroenteritis. Whereas CaCV possesses properties appropriate for the family Caliciviridae, it appears to be unrelated (or only distantly related) to other caliciviruses or presumptive caliciviruses including those from dogs. CaCV may be widespread among dogs, and though isolated from a dog with diarrhea its role as a causal agent of gastroenteritis has not been established.

**Materials and Methods**

*Virus and Cells*

Isolation of CaCV and its propagation in canine cells is described under Results. Several lines of dog kidney cells were propagated in medium MEM with 5 percent fetal bovine serum. These included MDCK cells, a greyhound kidney cell line (Flow Laboratories, Irving, Scotland), and a line designated DK (Beecham Laboratories, White Hall, IL). Dolphin kidney cells (NBL10, SP1K) were supplied by Flow Laboratories (Irving, Scotland). FCV strain F9 was isolated from a commercial vaccine (36), and strains LLK and 2280 were obtained from Niels Pedersen (23); all were propagated in CRFK feline cells. Poliovirus Type 1 strain LSc2ab was propagated in Vero monkey kidney cells. Virus assays were performed in microtiter plates as described (31).

*Purification*

Purification of CaCV is described in the Results section. FCV labeled with $^{32}$P (20 µCi/ml medium) was extracted from infected cells by a mixture of phospholipase C, DNase and RNase (68, 34 and 340 µg/ml, respectively, 1 hour, 37°C) and purified by centrifugation (40 minutes 45,000 rpm, 4°C in Spinco SW50.1 rotor) into a 10—32 percent glycerol gradient (w/w in PBS with 0.1 percent bovine serum albumin, BSA) as described (28). Poliovirus labeled with $^3$H-uridine was purified by pelleting virus from infected cell supernatant and banding in CsCl (22).

*Physico-Chemical Characterization*

Techniques for rate zonal centrifugation in glycerol gradients (29), and isopycnic centrifugation in CsCl (38) were similar to those previously described, except that