Isolation and characterization of caliciviruses from dogs with vesicular genital disease

R. A. Crandell

Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas, U.S.A.

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Summary. Two virus isolates, one from lesions of the vagina of a Bearded Collie and the other from the penis and prepuce of a Black Labrador, were partially characterized. The two viruses possessed the physicochemical properties, size and morphology of viruses belonging to the family Caliciviridae. The two isolates were shown by cross neutralization tests to be distinct from previously reported canine and feline caliciviruses. The viruses, isolated four years apart, are antigenically related. Additional studies are necessary to determine whether they are two distinct viruses or strains of another serotype belonging to the caliciviruses of the canine species.

Introduction

Caliciviruses have been isolated from several animal species with reproductive problems. In 1972, a calicivirus (SMSV) was first isolated from an aborting San Miguel sea lion during an investigation of reproductive failures in that species [13]. Later, another calicivirus was isolated from an aborted sea lion fetus [14]. Abortions have been reported in sows during severe outbreaks of vesicular exanthema, now eradicated from domestic swine herds [8]. Vesicular lesions and/or ulcers have occurred in calicivirus-infected pigs [16], sea lions [14], domestic cats [3], cheetahs [10], and dogs [5].

In 1982, a female dog, and in 1986, a male dog, were presented at two different veterinary clinics in Texas with vesicular lesions of the genitalia. Two serologically related caliciviruses unrelated to the previously reported canine calicivirus (CaCV) [12] and feline calicivirus (CFI) [3] were isolated in cell culture.

The purpose of the present communication is to report the isolation and partial characterization of these two calicivirus isolates from dogs with infections of the genitalia, and their unrelatedness to the feline caliciviruses and to a previously reported canine calicivirus.
Materials and methods

Cell cultures
MDCK, Vero, CRFK cell lines and dog kidney (obtained from J. W. Black [12]) cells were propagated with Eagle's minimal essential medium (MEM) with non-essential amino acids, glutamine, 0.5% lactalbumin hydrolysate and 10% fetal bovine serum (FBS). Maintenance medium for MDCK, Vero and CRFK cells was MEM with 2% FBS. Dog kidney (DK) cells were maintained with MEM with Hepes buffer [12]. All media contained 200 IU/ml penicillin, 200 μg/ml streptomycin and 50 μg/ml gentamicin.

Clinical specimens
Specimens for virus isolation were collected by scraping the vesicular lesions on the mucosa of the vagina of an adult Bearded Collie (82 CV) and the penis and prepuce of a 2-year-old Black Labrador (86 CV) with cotton swabs. The swabs were shipped to the laboratory in a stoppered tube under wet ice. Blood for serological testing was also collected from each animal.

Virus isolation
Each swab was placed in 2 ml of MEM, mixed on a vortex mixer and stored at -70 °C until testing. MDCK cell culture tubes were inoculated with 0.3 ml of specimen (82 CV) and incubated at 36 °C. After thawing, 0.3 ml (86 CV) was adsorbed on the monolayer of MDCK cells in a 25 cm² flask for 1 hour. The medium was replaced and the flask was incubated at 36 °C and observed daily for cytopathic changes (CPC).

Serologic studies
Canine calicivirus (CaCV) obtained from J. W. Black [12], antiserum supplied by Fort Dodge Laboratories, feline calicivirus (CFI) [3] and immune CFI antiserum were used. After three terminal dilutions of each isolate, immune serum was prepared in rabbits [3]. All sera were heat inactivated at 56°C for 30 minutes. The standard cross serum neutralization (SN) test using 100–200 TCID₅₀ doses of virus were performed in microtiter plates with MDCK cells. The relationship of the 3 canine viruses was also determined by the constant serum-virus dilution method in tubes of DK cells. The sera were all tested at a dilution of 1:5.

Virus characterization
Thin sectioning and negatively stained transmission electron microscopy was performed as described [4, 6]. The effects of lipid solvents, nucleic acid determination, heat and acid stability, and the effect of divalent cations were studied [2].

Results

Virus isolation
The initial cytopathic change (CPC) of 82CV was detected in MDCK cells 24 hours after inoculation and consisted of a few rounded and refractile cells. The affected cells became granular and most were released from the vessel surface. The cells and fluid were passed, and after 24 hours incubation, virus particles were demonstrated by negative contrast staining. Virus growth was erratic.