Detection of Neonatal Calf Diarrhea Virus, Infant Reovirus-Like Diarrhea Virus, and a Coronavirus Using the Fluorescent Virus Precipitin Test¹

MARTIN W. PETERSON,² REX S. SPENDLOVE,* AND ROSS A. SMART

Departments of Biology and Veterinary Science, Utah State University, Logan, Utah 84322

Received for publication 9 October 1975

Thirty-four calf and five infant fecal specimens were tested for the neonatal calf diarrhea virus (NCDV) and for the reovirus-like infantile diarrhea agent, respectively. The procedures used were the fluorescent virus precipitin test and immune electron microscopy. Fourteen of the calf stools contained detectable NCDV, and four of the five infant stools contained the reovirus-like human agent. Infectious NCDV was detected in four of the 34 calf fecal specimens when Madin-Darby bovine kidney cell cultures that had been inoculated with supernatant fluids from stool suspensions were stained with fluorescent antibody. The 20 calf stools that did not have detectable virus were examined for the bovine corona diarrhea virus. Coronavirus was found in two of these specimens.

Two serologically related reo-like viruses are major etiological agents causing diarrhea in infants (2, 5) and calves (9). The calf virus has generally been referred to as the neonatal calf diarrhea virus (NCDV).

Immune electron microscopy (IEM) (8), the fluorescent virus precipitin test (FVPT) (4), and the immunofluorescent cell assay (1) were used to determine if NCDV and the human reo-like virus are present in northern Utah.

Calf fecal specimens were randomly collected from scouring calves in beef and dairy herds in northern Utah. Human fecal samples were from sporadic cases of infant diarrhea.

Fluorescein-labeled and unlabeled rabbit NCDV antisera were obtained from C. A. Mebus of the Veterinary Science Department of the University of Nebraska at Lincoln. The two antisera were used, respectively, in the FVPT and in most of the IEM tests for detecting both the NCDV and the human reovirus-like agent. The FVPT titer of the conjugate was 1:160. The IEM titer of the unlabeled antiserum was 1:4,096.

The bovine coronavirus antisera were prepared in rabbits by Norden Laboratories, Inc., Lincoln, Nebr.

Human and calf fecal samples were diluted approximately 1:5 with 0.85% NaCl and centrifuged for 15 min at $850 \times g$. The supernatant fluid was then tested for the presence of virus.

The IEM procedure (8) consisted of mixing equal volumes of fecal suspension and antiserum in the wells of microtiter plates. Following 1 h of incubation at 37 C, a carbon-coated electron microscope grid was lowered into and removed from the sample mixture. After negative staining with 2% phosphotungstic acid, the grid was observed in an electron microscope for the presence of viral aggregates.

In the FVPT (4) equal volumes of fecal suspension and fluorescein-labeled antiserum that had been filtered (0.4- μ m pore size) were mixed and incubated at 37 C for 1 h. The mixture was then centrifuged at $700 \times g$ for 10 min after which each tube was gently shaken. A drop from each resuspended mixture was placed on a microscope slide and observed under oil immersion by using a Zeiss microscope equipped with a vertical illuminator containing a 60-W tungsten bulb. Fluorescing debris attached to the cover glass or the microscope slide was disregarded. Virus was considered to be present when free floating, fluorescing aggregates were observed.

In the immunofluorescent cell method (1), the fecal suspension was filtered and added to a monolayer of Madin-Darby bovine kidney cells. Twenty-four hours after infection the cells were fixed with acetone, stained with fluorescent antibody, and observed for the presence of infected (fluorescing) cells.

Specificity testing of the procedures has been described previously (1, 4).

Results obtained were identical when calf fecal samples were tested for NCDV by the IEM

¹ This article is Utah Agricultural Experiment Station Journal paper no. 3035.

² Present address: Affiliated Laboratories, White Hall, Ill. 62092.

and FVPT procedures. Of the 34 samples tested, 14 (41%) were positive. When tested by the immunofluorescent cell assay method, infectious virus was detectable in four of the 34 specimens (Table 1). The four positive immunofluorescent cell specimens were also positive by the IEM and the FVPT procedures.

The communicability of scours caused by NCDV was demonstrated when six calves obtained at an auction all scoured within a few days after purchase. Fecal specimens from all six calves contained large numbers of NCDV. Three of the calves died.

When 16 locally collected adult bovine serum samples were mixed individually with NCDV suspensions, all of the serum samples aggregated the virus and thus contained antibody against NCDV. The serum samples obtained from animals in northern Utah and southern Idaho ranged from 1:4 to 1:256 in IEM titers.

Coronavirus also causes diarrhea in calves (7). Using the FVPT and IEM procedures two of the 20 NCDV-negative samples in our study were found to contain coronavirus.

Samples from five diarrheic children were tested by IEM and FVPT for the presence of the human reovirus-like agent. Virus was detected in stools from four of the five children. The age range was from 11 to 24 months, and the virus was excreted through day 7 of the clinical symptoms.

A convalescent-phase serum from an infant had an IEM titer of 1:2,048 against the human reo-like virus.

This study produced two notable results. First, it demonstrated that the FVPT was useful in detecting three different diarrhea viruses and their antibodies. Second, it proved that these three viruses (NCDV, a bovine coronavirus, and the human reo-like virus) are present in northern Utah.

Several methods have been developed for detecting NCDV. These include the staining of segments of the infected small intestine, purification and examination of specimens by electron microscopy (6), fluorescent antibody staining of infected tissue culture cells (1), and the staining of infected cells present in fecal material. Detection of NCDV using sluffed, infected cells requires that the samples be quick-frozen if immediate testing is not possible. A sensitive and rapid method is needed for the screening of large numbers of specimens. This study shows that the FVPT fills this need.

On first examination of the two fecal specimens that contained coronavirus the FVPT gave positive results, whereas the IEM results

TABLE 1. Comparison of three procedures for detecting NCDV

Procedure ^a	No. of samples tested	No. of positive samples	Positive (%)
IEM	34	14	41
FVPT	34	14	41
IFC	34	4	12

^a IEM, Immune electron microscopy; FVPT, fluorescent virus precipitin test; IFC, immunofluorescent cell assay.

were negative. Upon reexamination by IEM, viral aggregates were observed in both specimens. Coronaviruses have an envelope and are not as easily observed by IEM as are viruses without envelopes. The FVPT should be especially valuable in detecting enveloped viruses.

The human reo-like virus was aggregated with anti-NCDV antiserum as well as with human reo-like convalescent serum in the IEM and FVPT procedures. These cross-reactions and the morphological data obtained in this study substantiate other reports that the human reo-like virus and NCDV are related (3).

The study was supported in part by grant 516-15-104 from the Cooperative State Research Service of the U.S. Department of Agriculture.

LITERATURE CITED

1. Barnett, B. B., R. S. Spendlove, M. W. Peterson, L. Y. Hsu, V. A. LaSalle, and L. N. Egbert. 1975. Immunofluorescent cell assay of neonatal calf diarrhea virus. *Can. J. Comp. Med.* 39:462-465.
2. Bishop, R. F., G. P. Davidson, I. H. Holmes, and B. J. Ruck. 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. *Lancet* 2:1281-1283.
3. Flewett, T. H., A. S. Bryden, H. Davies, G. N. Woode, J. C. Bridger, and J. M. Derrick. 1974. Relation between viruses from acute gastroenteritis of children and newborn calves. *Lancet* 2:61-63.
4. Foster, L. G., M. W. Peterson, and R. S. Spendlove. 1975. Fluorescent virus precipitin test. *Proc. Soc. Exp. Biol. Med.* 150:155-160.
5. Kapikian, A. Z., H. W. Kim, R. G. Wyatt, W. J. Rodriguez, S. Ross, W. L. Cline, R. H. Parrott, and R. M. Chanock. 1974. Reovirus-like agent in stools: association with infantile diarrhea and development of serologic tests. *Science* 185:1049-1053.
6. Mebus, C. A., N. R. Underdahl, M. B. Rhodes, and M. J. Twiehaus. 1969. Calf diarrhea (scours): reproduced with a virus from field outbreak. *Univ. Nebr. Res. Bull.* 233:2-16.
7. Stair, E. L., M. B. Rhodes, R. G. White, and C. A. Mebus. 1972. Neonatal calf diarrhea: purification and electron microscopy of a coronavirus-like agent. *Am. J. Vet. Res.* 33:1147-1156.
8. Vassal, J. H., and C. G. Ray. 1974. Serotyping of adenovirus using immune electron microscopy. *Appl. Microbiol.* 28:623-627.
9. Woode, G. N., and J. C. Bridger. 1975. Viral enteritis of calves. *Vet. Rec.* 96:85-88.