Comparison of Counterimmunoelectrophoresis and Electron Microscopy for Laboratory Diagnosis of Human Reovirus-Like Agent-Associated Infantile Gastroenteritis

L. SPENCE,* M. FAUVEL, R. PETRO, AND S. BLOCH

Department of Medical Microbiology, University of Toronto, Toronto, Ontario Canada M5G 1L5

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Counterimmunoelectrophoresis was compared with electron microscopy for detection of human reovirus-like agent in fecal specimens. Both tests gave very similar results.

In 1973, Bishop et al. (2) reported the presence of a virus, which they described as an orbivirus, in duodenal biopsy specimens from children with gastroenteritis in Australia. Subsequently, the same virus was reported from other countries and was given a variety of names: rotavirus, duovirus, infantile gastroenteritis virus, and human reovirus-like agent (HRVL). The latter name is used throughout this paper. The virus was shown to be of importance in the etiology of infantile gastroenteritis (4, 10).

Different methods were used to detect the virus in the feces of patients suffering from gastroenteritis. In 1973, Flewett et al. (5) demonstrated by electron microscopy (EM) reovirus-like particles in fecal suspensions from children suffering from gastroenteritis. This observation was confirmed by other investigators (3, 14). In further studies, Flewett et al. (6) demonstrated antigenic relationships between the human virus and a virus associated with diarrhea in newborn calves, Nebraska calf diarrhea virus (NCDV). The immune EM test was used for these studies. These viruses were also shown to be antigenically related by the complement fixation test (9).

Because of the difficulty and high cost of examining large numbers of stools by EM, other tests for HRVL agent and NCDV were investigated. Foster et al. (7) described a fluorescence precipitin test used to detect NCDV in fecal specimens from calves. The virus particles were aggregated by anti-NCDV conjugate and examined by epifluorescence microscopy. The authors claimed that the test was almost as sensitive as immune EM. The method was used subsequently for detection of NCDV in calves and for HRVL in infants (12). Banatvala et al. (1) used pig kidney cultures to detect HRVL agent by immunofluorescence.

In 1975, Spence et al. (13) reported the use of

the complement fixation and counterimmunoelectrophoresis (CIEOP) tests for detection of HRVL antigen in stools. In this study, in which the precipitin lines were not stained, only 50% of the stools were found positive by CIEOP. A suspension of the patient's feces was used as antigen, and the antiserum was obtained from rabbits that had been hyperimmunized with NCDV. In 1976, Middleton et al. (11) gave a detailed description of a CIEOP test for detection of HRVL antigen in fecal specimens. They prepared HRVL antibody in guinea pigs by immunizing the animals with highly purified HRVL agent. Tannic acid was used to stain the antigen-antibody precipitate in the agarose.

In the present study we compared the CIEOP method with negative-staining EM for detection of the HRVL agent in fecal specimens. The studies were carried out on fecal specimens from three sources: pediatric wards of a hospital in Port of Spain, Trinidad; a pediatric practice in Montreal, Quebec; and the pediatric ward of a hospital in Hamilton, Ontario. The specimens were collected from infants during the acute stage of infantile gastroenteritis. They were tested by CIEOP and EM.

The CIEOP test used was basically that described by Middleton et al. (11). The fecal specimens (about 10 to 20%, wt/vol) were suspended either in normal saline or in Eagle minimum essential medium with 0.5% lactalbumin hydrolysate. Rather than the guinea pig anti-HRVL antiserum used by Middleton et al. (11), hyperimmune rabbit anti-NCDV antiserum (CIEOP titer, 1:256) was used at a dilution of 1:16 (16 units of antibody). Rabbits were immunized with NCDV that was propagated in a continuous line of monkey kidney cells (BS-C1, *Cercopithecus aethiops*) and purified by centrifugation on a cesium chloride gradient. Also, the agarose-coated slides were washed overnight prior to staining with tannic acid. For

TABLE 1. Comparison of CIEOP and EM for detection of HRVL agent in feces (273 specimens)

EM	CIEOP	
	Positive	Negative
Positive	86	4
Negative	1	182

EM the grids were prepared as follows: a Formvar-carbon-coated grid was placed on top of a bacteriological agar plate with the coated side facing upwards. One drop of the fecal suspension was placed on top of the grid, and the liquid phase was allowed to diffuse into the agar. The grid was then stained with a drop of 2% phosphotungstic acid, pH 7.0, for 30 s. The excess fluid was removed from the grid with absorbent paper, and the grid was examined in a Philips 201 electron microscope.

Both methods were used to test 273 fecal specimens. The results are shown in Table 1. Assuming that examination of the specimens by EM gives the correct answer, the sensitivity of the CIEOP test was calculated by the method of Galen and Gambino (8) to be 95.5% and the specificity to be 99.3%. The Kendall coefficient of association (v) for the EM and CIEOP tests was calculated to be +0.959 (chi square = 250.8, $P < 0.0001$).

These results show the CIEOP test to be sensitive and reliable and to have a close association with the EM test. It is a valuable test for routine laboratory diagnosis and for epidemiological studies, because large numbers of specimens can be processed by this method. Similar results were obtained by Tufvesson and Johnsson (15) with a CIEOP test using guinea pig anti-NCDV antiserum. In two separate studies, they found that 94 and 100% of EM-positive stools were positive by CIEOP.

The use of NCDV antiserum instead of HRVL antiserum surmounts the problem of using fecal specimens as a source of vaccine for rabbits or guinea pigs. NCDV can be propagated in cell culture, whereas HRVL grows with difficulty or not at all in cell culture. The use of tannic acid for staining precipitin lines greatly increases the sensitivity of the CIEOP test.

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