The Diagnosis of Rotaviral Infection

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Rotavirus is a common cause of non-bacterial diarrhea during the colder months of the year. It was only recently, in 1969, that rotavirus was associated with neonatal diarrhea in calves (6,8). Since that time, rotavirus has been found to infect a wide variety of animals, including pigs, mice, lambs, foals, rabbits, goats, deer and guinea pigs (5).

Rotavirus infects the mature cells, or enterocytes, that line the small intestine. Immature enterocytes, unlike the mature cells, are resistant to rotaviral infection. These immature cells are located between the villi and migrate toward the tips of the villi as they mature. Mature enterocytes die and fall from the villi tips due to infection, and the uninfected immature cells are pushed to the tips of the villi prematurely. The resulting villi are short, blunt and often fused together.

In acute, rotaviral-infected animals, an increased total fecal output is observed, and a heavy loss of body fluids quickly leads to mass dehydration. Numerous rotavirus particles are shed during the first two days of infection, and the number sharply decreases just before the diarrhea ends, although diarrheitic stool contains virus throughout the illness (4). If the animal survives, cell regeneration usually takes place rapidly (1).

Rotaviral infection may predispose the intestinal tract to enteropathogenic *E. coli* (9, 12). Further alteration of the cells of the small intestine by *E. coli* may further delay the restoration of normal intestinal status or result in an increased mortality rate (3, 7). Simultaneous or concurrent rotaviral and transmissible gastroenteritis (TGE) viral infections have been produced in pigs (10), and combined enteric infections in calves have also been documented (13). Usually the mortality rate is higher in animals when you have an *E. coli* or coronavirus infection combined with a rotaviral infection (3).

There are different species of rotavirus that infect different animals, but all rotavirus species have a particular antigen in common. This common antigen is a part of the virus, and this antigen is recognized by antibodies specific for rotavirus. Because of this common antigen, it is possible to gain evidence for the presence of rotavirus by two means: (1) whole virus isolation and identification, and (2) viral antigen detection. Whole virus isolation techniques include electron microscopy and fluorescent antibody. Methods that detect viral antigen include agar-gel immunodiffusion, counter-immunoelectrophoresis, and enzyme-linked immunosorbent assay.

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Specimens

Gut and fecal specimens were collected from 253 scouring and non-scouring calves. The specimens were diluted, incubated and frozen until tested. Before testing, the specimens were clarified by centrifugation.

Fluorescent Antibody

The fluorescent antibody method requires the application of a dye-containing material, called a conjugate, to cells that have been exposed to gut and fecal material. This conjugate contains antibodies that are specific for rotavirus and tagged with the dye fluorescein. If rotavirus is present in the gut or fecal material, the inoculated cells become infected. The labeled antibodies then attach to rotavirus inside the cell cytoplasm; under ultra-violet light, the cells fluoresce, or glow in a manner that is detectable with the ultraviolet, or fluorescent microscope.

Agar-Gel Immunodiffusion

With the agar-gel immunodiffusion test an antigen-antibody precipitin reaction was observed in a semi-solid medium. First, melted agar was carefully poured onto a glass slide. After the agar hardened, six small wells were cut around a cut center well, all of them the same distance from each other and the center well. Antiserum (serum that contains antibodies against rotavirus antigen) was placed into the center well with four test samples and two control antigens in the outer wells. Rotavirus antigen and rotavirus antiserum diffused toward each other in the agar and formed a precipitin band when they met. If there was no rotavirus antigen present in the test samples, no precipitin band was formed.

Counterimmunoelectrophoresis

The counterimmunoelectrophoresis test is based on the property that slow-moving proteins, like immunoglobulins, will migrate towards a cathode (which carries a positive charge) while the virus antigen, being negatively charged, will migrate towards an anode (2). When an electric current is applied, the anodally migrating antigen is forced to move, through agar, into the cathodally moving antibody, producing an antigen-antibody precipitin band. This band was visually enhanced by various staining procedures. The number of precipitin bands observed varied with antigen preparation; less bands were seen when highly purified antigen was used.

Enzyme-Linked Immunosorbent Assay

In the enzyme-linked immunosorbent assay test rotavirus-specific antibodies were bound to an insoluble carrier surface, which was used to "capture" the rotaviral antigen in the specimen. The complex formed was detected by an enzyme-labeled specific antibody, and degradation of the enzyme substrate depended upon how much rotavirus antigen was present in the test specimen (11). Test results depended upon two assumptions: (1) that the antibody attached to the support yet maintained its rotavirus-specific activity, and (2) that the antibody complex could be linked to the enzyme with both antibody and enzyme being able to retain their respective activities (11). Theoretically simple, much work was required to standardize the technique for successful routine diagnostic testing.

Test Results

Number of positives based on the following testing procedures:

Fluorescent Antibody (FA) - 39
Agar-Gel Immunodiffusion (AGID) - 24
Counterimmunoelectrophoresis (CIEP) - 27
Enzyme-Linked Immunosorbent Assay (ELISA) - 54

Comparison of FA and ELISA	
FA positive only - 13	19.4%
ELISA positive only - 28	40.7%
Both positive - 26	38.8%
Comparison of AGID and FA	
AGID positive only - 13	25.0%
FA positive only - 28	53.8%
Both positive - 11	21.0%
Comparison of AGID and CIEP	
CIEP positive only - 70	29.4%
AGID positive only - 7	20.5%
Both positive - 17	50.0%

Summary

From these results, the ELISA method appears to be the most sensitive of the four techniques. Whether this is true or not will be determined when electron microscopy work on each sample is completed. It is possible that the ELISA test is measuring rotavirus antigen as well as other, non-rotaviral antigien. If so, this would account for the greater sensitivity of the test.

Further work is necessary to improve, and understand, all of these techniques as to how they can be applied to rotavirus detection. Our laboratory will continue to evaluate this, and other, virological testing procedures.

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