Evaluation of a Latex Agglutination Kit (Virogen Rotatest) for Detection of Bovine Rotavirus in Fecal Samples†

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The performance of the Virogen Rotatest latex agglutination test (LAT) was evaluated for detection of bovine rotavirus antigen. Sixty-three fecal samples from diarrheic calves were collected from November 1999 to May 2000 and screened by LAT, the Rotazyme II enzyme-linked immunosorbent assay (ELISA), and virus isolation (VI) followed by an anti-rotavirus fluorescent-antibody (FA) test to detect the presence of group A rotavirus antigen. Of the 63 samples screened by VI-FA, 33 (58%) tested positive for rotavirus antigen. When the results from the LAT were compared to those from VI-FA, the "gold standard" for detection of bovine rotavirus in fecal samples, the sensitivity and specificity were found to be 87.8 and 73.3%, respectively. Latex agglutination compared with ELISA (the reference method) showed 100% sensitivity and 96.3% specificity, and when ELISA was compared with VI, the sensitivity was 84.8% and the specificity was 73.3%. Latex agglutination is easy to perform in a short time and does not require expensive equipment or skilled personnel, and the reagents have long shelf lives. These factors make the LAT suitable and highly efficient for use in a clinical laboratory as a rapid screening test for bovine rotavirus.

Rotaviruses are nonenveloped viruses belonging to the genus *Rotavirus* in the family *Reoviridae*. They are the major causes of dehydration and diarrhea in young children and many animal species. Group A rotaviruses are the major causes of enteric disease in calves (11, 14, 18, 22, 23, 24). The classification of rotaviruses into serotypes is based on the identification of two outer proteins, VP4 and VP7 (16, 19). Both VP4 and VP7 elicit the production of neutralizing antibodies shown to be protective against bovine rotavirus (BRV) infection in vivo and in vitro (4, 7, 9). VP7 serotypes (G types) can now be identified by enzyme immunoassay incorporating VP7-specific neutralizing monoclonal antibodies (16, 18).

Several tests are used routinely in diagnostic laboratories for the detection of rotavirus in fecal samples. These include enzyme-linked immunosorbent assay (ELISA) (2, 5, 13), electron microscopy, virus isolation (VI), passive hemagglutination, immunoelectrophoresis, and latex agglutination assays (6, 8, 9, 10, 20).

In this study, we compared the Virogen Rotatest kit (Wampole Laboratories, Cranbury, N.J.), a latex agglutination test (LAT), with the Rotazyme II ELISA kit (Abbott Laboratories, Abbott Park, Ill.) and with VI for sensitivity and specificity of detection of BRV in fecal samples. VI followed by a fluorescent-antibody (FA) test was used as the "gold standard" method.

MATERIALS AND METHODS

Fecal collection and preparation. Sixty-three fecal specimens obtained from calves with acute gastroenteritis were submitted to the Veterinary Diagnostic Laboratory at Kansas State University, Manhattan, between November 1999 and May 2000. These cases were from Kansas and Nebraska. The negative and positive control samples were from healthy animals and from cell culture supernatants that tested positive, respectively. Fecal samples were prepared as either a 10% (wt/vol) suspension of solid or semisolid feces in 0.01 M phosphate-buffered saline (PBS; pH 7) or as a 20% (vol/vol) suspension of liquid feces in 0.01 M PBS (pH 7). All samples were centrifuged at $1,500 \times g$, and the supernatants were tested and then stored in sterile vials at $-80°C$ for further study.

Latex agglutination slide test. The LAT for rotavirus detection in bovine fecal samples was performed with the Virogen Rotatest kit following the manufacturer's instructions for human fecal samples. This is a rapid slide test in which latex particles are coated with antibodies specific for group A rotavirus antigens present in a fecal supernatant. This test is read with the naked eye in 5 min.

Virus isolation and immunofluorescence. The fecal sample supernatants were filtered through 0.45-μm-pore-size syringe filters, and the filtrates were collected in sterile 1.5-ml freezer vials (Sarstedt, Inc., Newton, N.C.). Flasks (25 cm) of monolayered embryonic bovine kidney (EBK) cells were washed with sodium- and magnesium-free PBS (pH 7), inoculated with 0.5 ml of filtered fecal supernatant (one specimen per flask), and then incubated for 1 h at 37°C in a 5% CO_2 incubator. This was followed by the addition of 5 ml of Eagle's minimum essential medium containing trypsin (5 μg/ml) and pancreatin (5 μg/ml) (1, 12). After two passages (inoculation of filtered virus onto monolayered EBK cells and incubation for 3 days, after which the virus supernatant was collected and another monolayer of EBK cells was infected and incubated for an addional 3 days), the supernatants were used to infect EBK cells in 48-well plates, which were incubated for 72 h at 37°C and 5% CO_2. The supernatants were collected again and used to infect EBK cells in Leighton tubes (Bellco Glass, Inc., Vineland, N.J.), which were incubated at 37°C for 48 h. FA testing was performed on the Leighton slides to confirm the presence of rotavirus. Briefly, the medium was removed from the Leighton tubes, stored in sterile vials at $-20°C$, and labeled appropriately for further study. The Leighton slides were washed with 0.01 M PBS (pH 7), and the cells were fixed in 2 ml of acetone at 4°C for 10 min. After the slides were air dried for 10 to 15 min, circles were drawn around the edges of the slides with a Marktext pen, a 1:30 dilution of goat anti-rotavirus fluorescein isothiocyanate-labeled antibody (NVSL, Ames, Iowa) was applied to each slide, and the slides were placed in a high-humidity 37°C incubator for 2 h. The slides were washed with 0.01 M PBS (pH 7), dried for 10 to 15 min at 37°C, mounted with buffered glycerol (pH 7.2), and then examined with a fluorescence microscope for the presence of a positive result, which indicates BRV infection.

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TABLE 1. Comparison of outcomes from three testing methods for BRV in 63 fecal samples[a]

Result of test[b]			Frequency (no. of specimens)	πi
LAT	ELISA	VI		
+	+	+	28	0.44
−	−	−	22	0.35
+	+	−	8	0.13
+	−	+	1	0.016
−	−	+	4	0.064

[a] Agreement was determined with reference to the total number of specimens tested ($n = 63$). Both the LAT and ELISA were used for detecting BRV in calf feces. πi = frequency/n, where n is the portion of samples having a particular outcome.

[b] +, positive; −, negative.

ELISA. The Rotazyme II kit, an assay appropriate for human diagnostic testing, was used in this study. Fecal samples were tested following the manufacturer's instructions for human fecal samples. The kit utilizes beads coated with guinea pig anti-rotavirus antiserum, PBS as a negative control, and simian rotavirus SA-11 as a positive control. A coated bead was added to each control sample and diluted fecal sample, and the samples were incubated for 1 h at 37°C. The beads were washed three times with sterile double-distilled water, and secondary rabbit anti-rotavirus horseradish peroxidase-labeled conjugate antibody was added. After incubation at 37°C for 1 h, the beads were washed three times with double-distilled H_2O, and then O-phenolethylene dihydrochloride was added. The developed color was compared to the color chart provided.

Calculation of specificity and sensitivity. The following formulas were used to calculate the specificity and sensitivity of the LAT compared to those of both ELISA and VI (26): (i) sensitivity = [TP/(TP + FN)] × 100, where TP is a true-positive result as determined by the reference assay and FN is a false-negative result; (ii) specificity = [TN/(TN + FP)] × 100, where TN is a true-negative result as determined by the reference assay and FP is a false-positive result.

RESULTS

Fecal samples from 63 calves with clinical diarrhea due to gastroenteritis were tested for BRV by the Rotatest latex slide agglutination test, the Rotazyme II ELISA, and VI in cell culture using fluorescently labeled anti-BRV antibodies. Rotavirus was detected in 36 specimens (57%) by ELISA, in 40 specimens (63%) by LAT, and in 33 specimens (52%) by VI-FA. The concordance of results among the three tests is shown in Table 1. Twenty-eight specimens (44%) were positive by all three assays, and 22 specimens (35%) were negative by all three assays. Latex agglutination and ELISA both detected eight samples (13%) as positive that VI-FA failed to identify. One sample (1.6%) was positive by latex agglutination and VI-FA but negative by ELISA. Four samples (6.4%) were positive by VI-FA only. The sensitivities and specificities (Table 2) of the Rotazyme II and Rotatest kits were calculated against the VI-FA gold standard. Both tests were 73.3% specific; however, the Rotatest LAT was 87.8% sensitive compared to the Rotazyme II ELISA, which was 84.8% sensitive. Comparison of latex agglutination to ELISA (the reference method) resulted in 100 and 96.3% for the sensitivity and specificity, respectively.

DISCUSSION

A rapid, simple, sensitive, and specific diagnostic technique for the detection of viral agents causing gastroenteritis is needed to facilitate timely treatment of the disease. Because rotavirus is a major agent associated with acute diarrhea in

TABLE 2. Sensitivity and specificity of latex agglutination compared with those of ELISA and VI as reference methods for detection of BRV infection[a]

Reference method	Assay	Sensitivity[b] (%)	Specificity[c] (%)
VI	LAT	87.8 (29/29 + 4)	73.3 (22/22 + 8)
	ELISA	84.8 (28/28 + 5)	73.3 (22/22 + 8)
ELISA	LAT	100 (36/36 + 0)	96.3 (26/26 + 1)

[a] $n = 63$.
[b] Sensitivity = [TP/(TP + FN)] × 100.
[c] Specificity = [TN/(TN + FP)] × 100.

human and animal species (24), various methods for the detection of rotavirus antigen in fecal samples have been developed. These include transmission electron microscopy, VI (3), ELISA (15, 17, 21), immunoassay, and latex agglutination (14, 23, 25).

Many factors, including laboratory size, number of specimens per day, and prevalence of the type of pathogen in that population, all influence the choice of protocols used for diagnostic testing. Transmission electron microscopy has been used in many diagnostic laboratories as the gold standard for virus detection. The electron microscope (EM) detects virus only if large numbers of particles are present (100,000 virus particles/g of feces) (20). It is expensive and requires special equipment and a specialized technician to operate it; thus, it is not suited for routine examination of many specimens, and we chose not to use EM in this study.

ELISAs are used widely in diagnostic laboratories because they provide rapid detection of rotavirus antigen in a relatively short time in comparison to other tests, such as VI. Commercial ELISAs, such as the Rotazyme II kit, are available for the routine diagnostic screening of large numbers of specimens. This kit is sensitive for the detection of rotavirus from several mammalian species, many of which possess the group A antigen, which is detected by Rotazyme II capture antibody. Most of the kits available on the market are designed primarily for humans and are not approved for veterinary diagnostic applications. We limited this evaluation of antigen detection to the use of ELISA and the LAT. The LAT is ideal for the number of specimens we receive daily to test for RNA viruses. ELISA is also good for laboratories handling large numbers of specimens on a daily basis; however, it is less sensitive than latex agglutination.

VI is the gold standard method for the detection of BRV because, when the concentration of virus particles is low, growth in one to three passages in cell culture results in amplification of the virus to levels that allow detection by anti-BRV FAs. Because low numbers of viruses can be amplified with repeated passages, VI is more sensitive than latex agglutination and ELISA. VI is routinely performed in most veterinary diagnostic laboratories, but some have difficulty growing BRV in tissue culture. The virus can be propagated in primary EBK cells; however, this is time-consuming, as it takes from 3 to 8 days for a cytopathic effect to develop for each passage.

In our study, we evaluated the Virogen Rotatest and Rotazyme II kits for the detection of BRV in clinical fecal samples and compared them to VI-FA, the gold standard. We found that the sensitivity and specificity of the kit were dependent on

which test it was compared with. For example, the Virogen Rotavirus kit showed higher sensitivity than ELISA (87.8 versus 84.8%) and specificity equal to that of ELISA (73.3%) when both commercial kits were compared with VI-FA. We expected the commercial kits to show higher sensitivity and specificity, since both are used for the detection of BRV. This may have been due to the low affinities of detecting antibodies or a low number of virus particles present in the specimen.

One specimen was positive by the LAT and VI but negative by ELISA. This suggests the presence of nonspecific factors interfering with ELISA. The higher sensitivity of the LAT may have also been a factor. Four specimens were negative by both ELISA and latex agglutination but positive by VI. This may be due to a low number of virus particles, which is not easy to detect by the LAT or ELISA. Eight specimens were positive by both the LAT and ELISA but negative by VI. The failure of VI may be due to inactivation of virus caused by contamination with bacteria and fungi and exposure to the physical and the chemical environment after specimen collection and during transport to the laboratory.

The results from this study show that the LAT is a valuable tool in the diagnosis of BRV infection. The assay has a number of advantages, including its simple format, rapidity, and low cost, and it can be performed without the need for trained personnel or expensive equipment (8). Both the LAT and ELISA are more sensitive than EM, and these assays take less time than VI (3, 6). The LAT showed higher sensitivity (87.8%) than ELISA (84.8%), while the specificity of the LAT correlates with that of ELISA. In addition, the LAT has the advantage that it can be read with the naked eye, making it easy to perform in every laboratory. Management of diarrheal diseases demands rapid, accurate diagnosis; therefore, the use of the LAT to detect viral antigen from diluted fecal samples is a good alternative to both VI and ELISA.

In conclusion, our study showed that latex agglutination is clearly a reliable and rapid method for the detection of BRV. Further study should be performed to develop an even more sensitive and specific latex agglutination assay for the diagnosis of BRV infection.

REFERENCES

1. Al-Yousif, Y., F. Al-Majhdi, C. Chard-Bergstrom, J. Anderson, and S. Kapil. 2000. Development, characterization, and diagnostic applications of monoclonal antibodies against bovine rotavirus. Clin. Diagn. Lab. Immunol. **7**:288–292.
2. Athanassious, R., G. Marsolias, R. Assaf, S. Dea, J. Descoteaux, R. Dulude, and C. Montpetit. 1994. Detection of bovine coronavirus and type A rotavirus in neonatal calf diarrhea and winter dysentery of cattle in Quebec: evaluation of three diagnostic methods. Can. Vet. J. **35**:163–169.
3. Benfield, D. A., I. J. Stotz, E. A. Nelson, and K. S. Groon. 1984. Comparison of a commercial enzyme-linked immunosorbent assay with electron micros-copy, fluorescent antibody, and virus isolation for the detection of bovine and porcine rotavirus. Am. J. Vet. Res. **45**:1998–2002.
4. Chen, D., and R. F. Ramig. 1992. Determinants of rotavirus stability and density during CsCl purification. Virology **186**:228–237.
5. Czeruy, C. P., and W. Eichhorn. 1989. Characterization of monoclonal and polyclonal antibodies to bovine enteric coronavirus: establishment of an efficient ELISA for antigen detection in feces. Vet. Microbiol. **20**:111–122.
6. De Beer, M., I. Peenze, V. M. De Costa, and A. D. Steele. 1997. Comparison of electron microscopy, enzyme-linked immunosorbent assay and latex agglutination for the detection of bovine rotavirus in faeces. J. S. Afr. Vet. Assoc. **68**:93–96.
7. Estes, M. K. 1996. Rotaviruses and their replication, p. 1625–1656. In B. N. Fields and D. M. Knipe (ed.), Field's virology, 3rd ed. Lippincott-Raven, Philadelphia, Pa.
8. Haikala, O. J., J. O. Kokkonen, and M. K. Leinonen. 1983. Rapid detection of rotavirus in stool by latex agglutination: comparison with radioimmunoassay and electron microscopy and clinical evaluation of the test. J. Med. Virol. **11**:91–97.
9. Hoshino, Y., and A. Z. Kapikian. 1994. Rotavirus antigens. Curr. Top. Microbiol. Immunol. **185**:179–227.
10. Kaminjolo, J. S., and A. A. Adesiyun. 1994. Rotavirus infection in calves, piglets, lambs and goat kids in Trinidad. Br. Vet. J. **150**:293–299.
11. Kapikian, A. Z., J. Flores, Y. Hoshino, R. I. Glass, K. Midthun, M. Gorziglia, and R. M. Chanock. 1986. Rotavirus: the major etiologic agent in severe infantile diarrhea may be controllable by a 'Jennerian' approach to vaccination. J. Infect. Dis. **153**:815–822.
12. Kapil, S., K. L. Richardson, C. Radi, and C. Chard-Bergstrom. 1996. Factors affecting isolation and propagation of bovine coronavirus in human rectal tumor-18 cell line. J. Vet. Diagn. Investig. **8**:96–99.
13. Lucchelli, A., S. Y. Kang, M. K. Jayasekera, A. V. Parwani, D. H. Zeman, and L. J. Saif. 1994. A survey of G6 and G10 serotypes of group A bovine rotaviruses from diarrheic beef and dairy calves using monoclonal antibodies in ELISA. J. Vet. Diagn. Investig. **6**:175–181.
14. Lucchelli, A., S. E. Lance, P. B. Bartlett, G. Y. Miller, and L. J. Saif. 1992. Prevalence of bovine group A rotavirus shedding among dairy calves in Ohio. Am. J. Vet. Res. **53**:169–174.
15. Mathur, M. S., and G. G. Bhave. 1993. Comparative evaluation of coagglutination and latex agglutination test (Rotalex kit) for detection of rotavirus. J. Postgrad. Med. **39**:130–131.
16. McNulty, M. S., D. Todd, G. M. Allan, J. B. McFerran, and J. A. Green. 1984. Epidemiology of rotavirus infection in broiler chicken: recognition of four serogroups. Arch. Virol. **81**:113–121.
17. Noorduyn, L. A., M. J. Meddens, J. Lindeman, W. C. Van-Dijd, and P. Herbrink. 1989. Favorable effect of detergent on antigen detection and comparison of enzyme linked detection systems in an ELISA for Chlamydia trachomatis. J. Immunoassay **10**:429–448.
18. Ofitt, P. A., R. D. Shaw, and H. B. Greenberg. 1986. Passive protection against rotavirus-induced diarrhea by monoclonal antibodies to surface protein VP3 and VP7. J. Virol. **58**:700–703.
19. Parwani, A. V., H. A. Hussein, R. I. Blair, A. Lucchelli, L. Navarro, and L. J. Saif. 1993. Characterization of field strains of group A bovine rotaviruses by using polymerase chain reaction-generated G and P type-specific cDNA probes. J. Clin. Microbiol. **31**:2010–2015.
20. Reynolds, D. J., D. Chasey, A. C. Scott, and J. C. Bridger. 1984. Evaluation of ELISA and electron microscopy for detection of coronavirus and rotavirus in bovine faeces. Vet. Rec. **114**:397–401.
21. Rouch, C. F., T. J. G. Raybould, and S. D. Acres. 1984. Monoclonal antibody capture-linked immunosorbent assay for detection of bovine enteric coronavirus. J. Clin. Microbiol. **19**:3883–3893.
22. Saif, L. J. 1991. Bovine rotavirus, p. 126–130. In A. E. Castro and W. P. Heuschele (ed.), Diagnostic veterinary virology: a practitioner's guide. The Williams & Wilkins Co., Baltimore, Md.
23. Sanders, R. C., A. D. Campbell, and J. C. Jenkins. 1986. Routine detection of human rotavirus by latex agglutination: comparison with enzyme linked immunosorbent assay, electron microscopy, and polyacrylamide gel electrophoresis. J. Virol. Methods **13**:285–290.
24. Theil, K. W. 1990. Group A rotaviruses, p. 35–72. In L. J. Saif and K. W. Theil (ed.), Viral diarrheas of man and animals. CRC Press, Inc., Boca Raton, Fla.
25. Thomas, E. E., D. L. Roscoe, L. Book, B. Bone, L. Browne, and V. Mah. 1994. The utility of latex agglutination assays in the diagnosis of pediatric viral gastroenteritis. Am. J. Clin. Pathol. **101**:742–746.
26. Vanrompay, D., A. Van Nerom, R. Ducatelle, and F. Haesebrouck. 1994. Evaluation of five immunoassays for detection of Chlamydia psittaci in cloacal and conjunctival specimens from turkeys. J. Clin. Microbiol. **32**:1470–1474.