Acta Scientiae Veterinariae, 2012. 40(1): 1014.

ORIGINAL ARTICLE
Pub. 1014

ISSN 1679-9216 (Online)

Evaluation of a Latex Agglutination Kit for Detecting Rotavirus in Piglets

Suelen Paesi, Caciano Elonir da Rosa, Phelipe Rodrigues Marocco Dornelles, Felipe da Luz, Cesar Augusto Schenkel & Denise Zampieri

ABSTRACT

***Background*:** The group A rotavirus is the most important ethiological agent that causes diarrhea in newborn humans and animals. They belong to the *Reoviridae* family and have a genome consisting of 11 segments of double-stranded RNA enclosed in a triple-layered capsid. Rotaviruses are classified in six groups (A to G) based on the VP6 capsid protein or on the migration pattern of genomic segments in polyacrylamide gel. Groups A, B, and C were found either in humans or in animals, while groups D to G were found only in animals. The outer layer is formed by two proteins, VP7 and VP4, which elicit neutralizing antibody responses and form the basis of the current dual classification system in G (VP7) and P (VP4) types. The early diagnostic of this viral infection in raising farms is crucial for preventing the dissemination of the disease among the animals. Latex agglutination tests (LAT) are quick and easily done, and require low financial investments.
***Materials, Methods & Results*:** The present study evaluated the *Rotavirus Latex*® kit (Richmond Immunosystems Diagnostics) currently used with humans for detecting the presence of rotavirus antigen in diarrheic feces of piglets. In order to confirm the results obtained with the LAT, samples were analyzed by EIA with the *Ridascreen rotavirus*® kit (R-Biopharm). The results were compared with the rotavirus determination test by polyacrylamide gel electrophoresis (PAGE). During March 2007 and March 2008, 328 excrement samples of diarrheic piglets were collected at a hog-raising farm in the state of Rio Grande do Sul, Brazil. Results showed that the rotavirus was present in 38 samples (11.6%) assayed with LAT, while PAGE detected the virus in 26 samples (7.9%). This means that 15 positive cases (4.6%) were not identified by PAGE. This difference shows the higher sensitivity of LAT against PAGE. LAT showed 88.5% of sensitivity, 95% of specificity, 60.5% of positive predictive value, 99% of negative predictive value and 94.5% of accuracy.
***Discussion*:** 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 and a small farm as a rapid screening test for piglet rotavirus. A test used for detecting the etiological agent of gastroenteritis should be fast, easy to use, and specific for anticipating the disease treatment, thus minimizing unnecessary expenses and establishing prophylactic measures to protect the entire flock. The most important factor in choosing the method is the number of samples that should be collected, the qualification of the analysis laboratory, and the type of pathogen that has contaminated the flock. The diagnostic methods most easily applied are based on detecting viral particles or RNA from fecal samples. Most kits available in the market for detecting rotaviruses are designed for humans and are not adequate for use in veterinary diagnosis. Therefore, the results obtained herein presented high sensitivity and specificity for LAT, showing to be a valuable tool for diagnosing rotaviruses in pig feces. This system used in clinical laboratories might also be used in intensive animal farming systems or by small-scale pig raisers in a cooperative system.

Keywords: gastroenteritis, rotavirus, swine, diagnostic, latex agglutination.

Received: August 2011 www.ufrgs.br/actavet Accepted: November 2011

Laboratório de Diagnóstico Molecular, Instituto de Biotecnologia da Universidade de Caxias do Sul, RS, Brazil. CORRESPONDENCE: S. Paesi [sopaesi@ucs.br - FAX +55 (54) 3218-2149]. Rua Francisco Getúlio Vargas, n. 3120. CEP 95070-560 Caxias do Sul, RS, Brazil.

INTRODUCTION

Rotaviruses are the major cause of gastroenteritis in children and young animals of various species worldwide [15]. They belong to the *Reoviridae* family and have a genome consisting of 11 segments of double-stranded RNA enclosed in a triple-layered capsid. Rotaviruses are classified in six groups (A to G) based on the VP6 capsid protein or on the migration pattern of genomic segments in polyacrylamide gel. Groups A, B, and C were found either in humans or in animals, while groups D to G were found only in animals [9,15]. The outer layer is formed by two proteins, VP7 and VP4, which elicit neutralizing antibody responses and form the basis of the current dual classification system in G (VP7) and P (VP4) types [8,9].

In domestic and confined animals, the rotavirus has a great involvement in clinical diseases, causing an increase in morbidity and mortality in young animals [20]. An increasing hog production requires the use of new management techniques to prevent the dissemination of pathogenic [7,16].

Several techniques have been developed for diagnosing rotavirus in feces. The detection of the viral agent was performed by electronic microscopy, polyacrylamide gel electrophoresis (PAGE), immunofluorescence, radioimmuneassay, reverse passive hemagglutination, enzyme immunoassays (EIA), latex agglutination test (LAT), and more recently by reverse transcriptase with polymerase chain reaction. Among these assays, LAT was reported as a being easy to perform in a short time, for diagnosis and control of the disease caused by rotavirus A in humans [4,10,11,13,17,19].

The purpose of the present study was to evaluate the potential of the Rotavirus Latex® kit for human use for diagnosing rotavirus in the feces of diarrheic piglets.

MATERIALS AND METHODS

A total of 329 fecal samples were collected from diarrheic piglets from a farm located in the mountain region of the state of Rio Grande do Sul, Brazil. Piglets age was around 40 days. The samples were collected from March 2007 to March 2008, sent to the Laboratory of Molecular Diagnostic of the University of Caxias do Sul, and stored at -20°C until use.

The samples were tested with the *Rotavirus Latex*® kit[1] according to the manufacturers' instructions. This is a rapid slide test in which latex particles are coated with antibodies specific for group-A rotavirus antigens present in fecal supernatant. The test was considered positive for rotavirus if agglutination was observed with the test latex, but not with the control latex, and undetermined if agglutination was observed with the test latex and the control latex. In order to confirm the results obtained with the LAT, samples were analyzed by EIA with the *Ridascreen rotavirus*® kit[2] according to the manufacturers' instructions. The EIA is an immunoassay with sandwich-type monoclonal antibodies that link to the VP6 protein of the rotavirus capsid.

To characterize the RNA electrophoretic pattern of the rotavirus, viral RNA was extracted from fecal specimens using phenol-chloroform [3]. The extracted RNA was subjected to polyacrylamide gel electrophoresis to detect viral genome and to characterize the RNA electrophoretic pattern described previously [25]. PAGE was performed using a 7.5% polyacrylamide separating gel with a 3.5% stacking gel, and electrophoresis was carried out for 8 h at a constant current of 20 mA per gel. RNA extracted from simian rotavirus (SA11) was used as positive control for PAGE analysis. Migration of RNA genome fragments was detected by silver staining [12,23].

The data surveyed in interviews and analytical results were tabulated in the *Statistical Package for the Social Science* (SPSS) - version 15.0 - with a significance level of $P < 0.05$ for statistical analysis.

RESULTS

Results showed that the rotavirus was present in 38 samples (11.6%) assayed with LAT, while PAGE detected the virus in 26 samples (7.9%) [Table 1]. This means that 15 positive cases (4.6%) were not identified by PAGE. This difference shows the higher sensitivity of LAT against PAGE. On the other hand, of the 291 samples with a negative result by LAT, three (0.9%) had a positive result with PAGE. Both methods have simultaneously identified 288 (87.5%) negative cases, characterizing a rotavirus prevalence of 12.5%.

When comparing LAT with PAGE, results obtained herein showed 88.5% of sensitivity, 95.0% of specificity, 60.5% of positive predictive value, 99.0% of negative predictive value and 94.5% of correlation (Table 1).

Table 1. Comparison between results obtained by latex agglutination (LAT) and polyacrylamide gel electrophoresis (PAGE) in 329 samples of diarrheic feces of pigs.

	PAGE		
LAT*	Positive	Negative	Total
Positive	23 (7%)	15 (4.6%)	38 (11.6%)
Negative	3 (0.9%)	288 (87.5%)	291 (88.4%)
Total**	26 (7.9%)	303 (92.1%)	329 (100%)

*88.5% sensitivity, 95% specificity, 60.5% positive predictive value, 98.9% negative predictive value, 94.5% accuracy. **Chi-square test was applied to the results obtained with LAT and PAGE; significant difference was $P < 0.005$.

DISCUSSION

A test used for detecting the etiological agent of gastroenteritis should be fast, easy to use, and specific for anticipating the disease treatment, thus minimizing unnecessary expenses and establishing prophylactic measures to protect the entire flock. The most important factor in choosing the method is the number of samples that should be collected, the qualification of the analysis laboratory, and the type of pathogen that has contaminated the flock. The diagnostic methods most easily applied are based on detecting viral particles or RNA from fecal samples [12,18,23,26,27]. Therefore, LAT and PAGE are the methods of choice in hog raising farms. PAGE is a method that has high specificity but low sensitivity. Its major advantage is detecting rotaviruses of several types [9,12]. On the other hand, although detecting only the type-A rotavirus, LAT has a high sensitivity and specificity, and it is widely used in diagnostic labs for its ability in detecting antigens in a short time and at a low cost [5,26]. However, LAT is not commercialized as a kit for use with hog herds.

The presence of rotavirus in hog herds is well known. Calderaro *et al*. [6] studied the frequency of etiological agents of enteritis in suckling piglets from 21 hog raising farms in Sao Paulo between March 1996 and November 1997. The authors used PAGE and found a 10.9% frequency of rotavirus amongst other bacterial and parasitological causative agents of gastroenteritis. A study conducted in Poland with 117 fecal samples from diarrheic piglets used PAGE and obtained 21 positive samples (17.9%) for rotavirus [29]. This is higher than the results reported herein; however, it might be explained by differences in handling, viral load, and geographical circulation of the etiological agent.

Among the limitations of the LAT, one must take into account the fact that this test detects only the type-A rotavirus, despite this being the most prevalent viral group in gastroenteritis of various etiologies. Janke *et al*. [14] when studying 90 fecal samples from piglets, found 67.8% of infections by type-A, 10% for type-B, and 11.1% for type-C rotaviruses. Pongsuwanna *et al*. [24] when studying 557 fecal samples from diarrheic piglets in Thailand using PAGE, identified 23 samples with type-A, one with type-B, and two with type-C rotaviruses. Another study conducted by Will *et al*. [28] reported that 89% of diarrhea cases in commercial raising farms are due to the type-A rotavirus.

Despite its high specificity, PAGE requires a long time investment when compared to the LAT, a drawback in case of epidemic outbreaks [12]. Latex agglutination tests are usually very popular in clinical laboratories. These tests have already been used for detecting over 100 different infectious diseases in humans, as in recent examples: malaria, tuberculosis, hepatitis C, and dengue fever [21]. A LAT kit for human use has already been successfully applied to bovine diarrheic samples and showed a 63% positive rate for type-A rotavirus [1]. The rate of false negatives (0.9%) by LAT and refuted by PAGE might be explained by the fact that the samples contained different types of rotaviruses, which the type-A rotavirus kit was unable to identify. Other kits also show this limitation, as for example Rotalex from Orion Diagnostica [17] with 2.7%, and Slidex Rotatest [13] with 2.4%. These false negative reactions probably occur due to a lower viral titer than the technical sensitivity or feces containing inhibitors or IgA-specific antibodies, resulting in weak agglutination reactions not detected by the kit [4,13]. Among the advantages of PAGE are the ability of this method in differentiating the electrophoretic profiles of the rotavirus present in the pig herd; however, the results of the present study show that 100% of the rotaviruses were classified as being of the long type, which means a greater presence of type-A rotavirus.

Group A is the target of the LAT kit for detecting rotaviruses in human fecal samples.

Other comparisons were conducted with humans, as for example between the *Slidex Rotavirus2*® (BioMérieux) kit and PAGE [11], where LAT showed 82.9% of sensitivity, 98.1% of specificity, 96.7% of positive predictive value, 89.7% of negative predictive value and 92.0% of accuracy. In another study comparing the PAGE technique with a LAT kit, Paul *et al*. [22] obtained a 70.9% sensitivity and a100% specificity. Altindis *et al*. [2] when studying feces samples from 135 diarrheic children reported a positive rate for rotavirus of 15.5% (21 samples) with LAT and of 11.8% (16 samples) with PAGE. When using PAGE as standard, they obtained 93.7% of sensitivity, 94.9% of specificity, 71.4% of positive predictive value, 99.1% of negative predictive value and 94.8% of accuracy for LAT.

CONCLUSIONS

Most kits available in the market for detecting rotaviruses are designed for humans and are not adequate for use in veterinary diagnosis. Therefore, the results obtained herein presented high sensitivity and specificity for LAT, showing to be a valuable tool for diagnosing rotaviruses in pig feces. This system used in clinical laboratories might also be used in intensive animal farming systems or by small-scale pig raisers in a cooperative system.

SOURCES AND MANUFACTURERS

[1]Rotavirus Latex® kit, Richmond Immunosystems Diagnostics Ltda. São Paulo, Brazil.

[2]Ridascreen rotavirus® kit, R-Biopharm. Darmstadt, Germany.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 Al-Yousif Y., Anderson J., Chard-Bergstrom C. , Bustamante A., Muenzenberger M., Austin K. & Kapil S. 2001. Evaluation of a latex agglutination kit (Virogen Rotatest) for detection of bovine rotavirus in fecal samples. *Clinical and Diagnostic Laboratory Immunology*. 8: 496-498.

2 Altindis M., Yavru S., Simsek A., Ozkul A., Ceri A. & Hasan K.O.C. 2004. Rotavirus Infection in Children with Acute Diarrhea as Detected by Latex Agglutination, ELISA and Polyacrylamide Gel Electrophoresis. *Indian Pediatrics*. 41(4): 590-594.

3 Boom R., Sol C.J.A., Salimansm M.M.M., Jansen C.L., Wertheim-Van P.M.E. & Noordaa-Van Der J. 1990. Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology*. 28(3): 495-503.

4 Buser J., Risch L., Rutz T., Manang S. & Munzinger J. 2001. Comparison of a rotavirus latex agglutination test with two rapidimmunochromatographic test devices for detection of rotavirus in human feces. *European Journal of Clinical Microbiology & Infectious Diseases*. 20(4): 295- 296.

5 Brandt C. D., Arndt C.W., Evans G. L., Kim H.W., Stallings E. P., Rodriguez W.J. & Parrott R.H. 1987. Evaluation of a Latex Test for Rotavirus Detection. *Journal of Clinical Microbiology*. 25(9): 1800-1802.

6 Calderaro F.F., Baccaro M.R., Moreno A.M., Ferreira A.J.P, Jerez A.J. & Pena H.J.F. 2001. Freqüência de agentes causadores de enterites em leitões lactantes provenientes de sistemas de produção de suínos do estado de São Paulo. *Arquivo do Instituto Biológico* 8: 29-34.

7 Dewey C.E., Wittum T. E, Hurd H. S. Dargatz D.A. & Hill G.W. 1995. Herd and litter-level factors associated with the incidence of diarrhea morbidity and mortality in piglets 4-14 days of age. *Journal of Swine Health and Production*. 3: 21-28.

8 Estes M.K. & Cohen J. 1989. Rotavirus gene structure and function. *Microbiology and Molecular Biology Reviews*. 53(4): 410-449.

9 Estes M.K. 2001. Rotaviruses and their replication. In: Knipe D.M., Howley P.M., Griffin D.E., Lamb R.A., Martin M.A., Roizman B. & Strais S.E. (Eds.) *Fields Virology*. 4th edn. Philadelphia: Lippincott Williams and Wilkins, pp.1747-1785.

10 Fernandes J.V. 2000. Rotavirus detection in feces of children with acute diarrhea. *Journal of Pediatrics*. 76: 300-304.

11 Ferreira T.L., Becho M.C., Bernardo A.R., Chaves T.C.B., Ribeiro R.S., Lima J.S., Fialho A.M., Leite J.P.G., Mazur C. & Anelli M.G.M. 2006. Performance of latex agglutination test in the diagnosis of acute gastroenteritis by rotavirus. *Brazilian Journal of Microbiology*. 37: 587-589.

12 Herring A.J., Inglis N.F., Ojeh C.K., Snodgrass D.R. & Menzies J.D. 1982. Rapid diagnosis of rotavirus infection by directdetection of viral nucleic acid in silver-stained polyacrylamide gel. *Journal of Clinical Microbiology*. 16(3): 473-477.

13 Ibrahim O.S., Sunderland D. & Hart C.A. 1990. Comparison of four methods for detection of rotavirus in feces. *Tropical Doctor*. 20(1): 30-32.

14 Janke B.H., Nelson J.K., Benfield D.A. & Nelson E.A. 1990. Relative prevalence of typical and atypical strains among rotaviruses from diarrheic pigs in conventional swine herds. *Journal of Veterinary Diagnostic Investigation*. 2: 308-311.

15 Kapikian A.Z., Hoshino Y. E & Chanock R.M., 2001. Rotaviruses. In: Knipe D.M., Howley P.M., Griffin D.E. Lamb R.A., Martin M.A., Roizman B. & Strais S.E. (Eds.). *Fields Virology*. 4th edn. Philadelphia: Lippincott Williams and Wilkins, pp.1787-1833.

16 Katsuda K., Kohmoto M., Kawashima K. & Tsunemitsu H. 2006. Frequency of enteropathogen detection in suckling and weaned pigs with diarrhea in Japan. *Journal of Veterinary Diagnostic Investigation*.18: 350-354.

17 Lipson S.M. & Zelinsky-Papez K.A 1989. Comparison of four latex agglutination (LA) and three enzyme-linked immunosorbent assays (ELISA) for the detection of rotavirus in fecal specimens. *American Journal of Clinical Pathology*. 92(5): 637-643.

18 Lucchelli A., Lance S. E., Bartlett P.B., Miller G. Y. & Saif L.J. 1992. Prevalence of bovine group A rotavirus shedding among dairy calves in Ohio. *American Journal of Veterinary Research*. 53: 169-174.

19 Marshall J., Botes J.; Gorrie G., Boardman C., Gregory J., Griffith J., Hogg G. Dimitriadis A., Catton M. & Bishop R. 2003. Rotavirus detection and characterization in outbreaks of gastroenteritis in aged-care facilities. *Journal of Clinical Virology*. 28(3): 331-340.

20 Mcnulty M. S. 1983. Rotavirus infections in calves. *Annals of Veterinary Research*. 14(4): 427-432.

21 Molina-Bolívar J.A. & Galisteo-González F. 2005. Latex immunoagglutination assays. *Journal of Macromolecular Science Polymer Reviews*. 45: 59-98.

22 Paul S.K., Tabassum S., Islam M.N., Ahmed M.U., Haq J.U. & Shamsuzzaman A.K. 2006. Diagnosis of human rotavirus in stool specimens: comparison of different methods. *Mymensingh Medical Journal*. 15(2): 183-187.

23 Pereira H.G., Azeredo R.S., Leite J.P.G., Candeias J.A.N., Rácz M.L., Linhares A.C., Gabbay Y.B. & Trabulsi L.R. 1983. Electrophoretic study of the genome of human rotaviruses from Rio de Janeiro, São Paulo and Pará, Brazil. *Journal Hyg-Cambridge*. 90: 117-125.

24 Ponguwanna Y., Taniguchi K., Chiwakul M., Urasawa T., Wakasugi F., Jayavasu C.E & Urusawa S. 1996. Serological and genomic characterization of porcine rotaviruses in Thailand: detection of a G10 porcine rotavirus. *Journal of Clinical Microbiology*. 34(5): 1050-1057.

25 Rodger S.M. & Holmes I.H. 1979. Comparison of the genomes of simian, bovine, and human rotaviruses by gel electrophoresis and detection of genomic variation among bovine isolates. *Journal of Virology*. 30(3): 839-846.

26 Smith T.F., Wold A.D., Espy M.J. & Marshall W.F. 1993. New developments in the diagnosis of viral diseases. *Infectious Disease Clinics of North America*. 7(2): 183-201.

27 Thomas E.E., Roscoe D.L., Book L., Bone B., Bowne L. & Mah V. 1994. The utility of latex agglutination assays in the diagnosis of pediatric viral gastroenteritis. *American Journal of Clinical Pathology*. 101: 742-746.

28 Will L.A., Paul P.S., Proescholdt T.A., Aktar S. N., Flaming K. P., Janke B.H., Sacks J., Lyoo Y.S., Hill H.T., Hoffman L.J. & Wu L.L. 1994. Evaluation of rotavirus infection in diarrhea in Iowa commercials pigs based on an epidemiologic study of a population represented by diagnostic laboratory cases. *Journal of Veterinary Diagnostic Investigation*. 6(4): 416-422.

29 Winlarczyk S. & Gradzki Z. 1999. Comparison of Polymerase Chain Reaction and Dot Hybridization with Enzyme-Linked Immunoassay, Virological Examination and Polyacrylamide Gel Electrophoresis for the Detection of Porcine Rotavirus in Faecal Specimens. *Journal of Veterinary Medicine, Series B*. 46(9): 623-634.