Evaluation of a Latex Agglutination Kit for Detecting Rotavirus in Piglets

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ABSTRACT

Background: The group A rotavirus is the most important etiological 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.
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, radioimmunoassay, 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 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 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).
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. Pongsuwanagna 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 a 100% 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


