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Latex Test for Rapid Rotavirus Diagnosis in Calves

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Sukura, A. and E. Neuvonen: Latex test for rapid rotavirus diagnosis in calves. Acta vet. scand. 1990, 31, 1-4. - A latex agglutination test (LA) was compared with direct electron microscopy (EM) for detection of rotavirus infection in calves. A total of 375 samples from 62 calves were collected. Samples were taken when the calves were 1, 3, 5, 7, 10 and 20 days old and some scours samples were collected as well. Altogether 45/375 (12%) specimens were positive in LA and 10/375 (2.7%) were positive in EM. Samples positive in EM were also positive in LA. Out of the 62 calves studied 26/62 (42%) were positive in LA and 8/62 (13%) in EM. We found the LA very easy to perform, to be more sensitive than the EM method and probably a rather specific method for detection of rotavirus infection.

latex agglutination; electron microscopy; calf diarrhoea.

Introduction

Rotavirus is an important causal agent of gastroenteritis in calves, either alone or together with some other enteropathogens (*McNulty* 1983, *Hess et al.* 1984, *Moon et al.* 1978, *Pohjola et al.* 1986 and *Tzipori et al.* 1983).

The methods for rotavirus diagnosis are the detection of the antigen in stool samples, visualization of virions in electron microscopy (EM) or the demonstration of an increase in antibody in serum samples.

In terms of viral concentration, 10^5-10^6 virions/ml is the minimum concentration which can be detected by the EM technique (*Davies* 1982). Clinically affected animals have been reported to secrete much more virus, even 10^{10} virions/g faeces (*McNulty* 1978). In these cases it is possible to develop rapid methods for virus detection. EM examination has been the standard method, and other methods have been compared to that (*Morinet et al.* 1984).

Latex agglutination (LA) is a simple modification of the reversed passive hemagglutination test (RPHA), where erythrocytes have been compensated by latex particles (*Sanekata et al.* 1981). In the LA-test, latex particles are covered by antirotavirus immunoglobulins. When the test suspension is mixed with a positive faeces suspension, rotavirus antigens visibly agglutinate the latex particles. All rotaviruses, except pararotavirus have common group antigens (*Chasey & Davies* 1984). It is therefore possible to use latex particles prepared for one species for the detection of rotavirus in the faeces of another species. Different subgroups may cause variations in sensitivity when testing stool specimens with heterologous viruses.

In this paper we compared a commercial LA test (Rotalex, Orion Diagnostica, Finland) with the direct negative-staining EM method for the detection of rotavirus in feces from calves. The antibodies for the LA test have been prepared by immunizing rabbits with

Nebraska calf diarrhoea virus and control latex has been prepared from the serum of unimmunized rabbits. The sensitivity of Rotalex has been evaluated to detect 30-100 ng human rotavirus protein/ml faecal suspension (*Haikala et al.* 1983).

Material and methods

Sample source

A total of 375 stool samples were collected from 8 different farms. Samples were from 62 individual calves. Samples were taken when the calves were 1, 3, 5, 7, 10 and 20 days old. From every calf complete sample series were not available for laboratory examination, and from some calves more samples than planned were received (scours samples). Historical data were collected as well. Samples were stored at -20°C.

Preparation of stool samples

The stool samples were diluted to 10% suspensions in phosphate buffered saline (pH 7.3) and centrifuged for 30 min at 600 g. One part of the supernatants was used for the LA test and the rest was stored at -20°C for EM-examination.

LA test

The LA test was performed according to the manufacturers instructions. Two drops of the sample were placed on a blackside glass slide. A drop of test reagent was added to the first drop and the negative control reagent was added to the other drop. Drops were mixed with a wooden stick. The glass slide was tilted manually and the reaction was read after 2 min.

The sample was considered positive for rotavirus if the negative control remained milky and the test sample showed distinct agglutination. If the negative control reagent agglutinated, the result was considered negative.

Electron microscopy

Frozen suspensions were melted and centrifuged for 20 min at 600 g. One drop of supernatant was placed on a carbon coated electronmicroscopy grid. Negative staining performed using 2% potassium phosphotungstate (pH 5.5). Grids were examined with a JEOL-JEM 100 S electron microscopy at a 40,000 magnification. All positive and uncertain particles were photographed and checked. Negative samples were studied for at least 15 min before they were regarded as negative.

Results

On the total of 375 samples examined, 45 (12%) were positive in the LA test and 10 (2.7%) were positive using the EM method (Table 1). Of the total number of 62 calves studied with both methods 26 (42%) of them were positive in LA test and by EM viruses were found in stool specimens of 8 (13%) calves (Table 2).

Table 1. Comparison of the LA test with the EM method in stool samples.

EM test	LA test		Total
	+	-	
+	10	0	10
-	35	330	365
Total	45	330	375

Table 2. Comparison of the LA test with the EM method in calves.

EM test	LA test		Total
	+	-	
+	8	0	8
-	18	36	54
Total	26	36	62

All samples positive by EM were also positive in the LA test. However, 35 samples were positive in the LA test but not by EM. Rota-

virus infections were detected in all 8 farms using the LA test, but in only 3 farms by EM. During the sampling period there were clinical diarrhoea in 42% of the LA positive calves and in 63% of the EM-positive calves. Of all the calves studied 36% had clinical manifestations of diarrhoea (Table 3).

Table 3. Comparison of the positive result with clinical diarrhoea.

	Clinical diarrhoea	Without clin. diarrhoea
Calves pos. in EM test	63% (5/ 8)	37% (3/ 8)
Calves pos. in LA test	42% (11/26)	58% (15/26)

Discussion

When comparing different diagnostic methods one should consider at least 4 factors: sensitivity, specificity, simplicity and economy of the method.

The reliability of the EM technique is at least affected by the concentration and purity of the specimen, the wetting properties of the grids, the time used in searching for virus in samples and operator skill (*Morinet et al.* 1984). In this work samples were not ultracentrifuged, and this makes the EM procedure less sensitive.

More positive results were obtained with the LA test than by EM. This indicates that LA may by more sensitive than EM, or less specific. This is in accordance with reports on the human rotavirus, where the LA test has proved to be as sensitive or more sensitive than the EM-method (*Morinet et al.* 1984, *Haikala et al.* 1983, *Pai et al.* 1985, *Julkunen et al.* 1985 and *Bryden* 1985). However, *Miotti et al.* (1985) found it less sensitive. There are also a few reports where comparisons have been performed with samples from domestic animals. *Goyal et al.* (1987) used bovine, porcine and turkey faecal samples for comparison of different methods. They found that the LA results were more sensitive in bovine samples than in turkey and porcine. Generally, they found the EM method more sensitive than the LA test, but in their work samples were ultracentrifuged for the EM procedure.

Subclinical rotavirus infections are common (*Crouch & Acres* 1984). Clinically sick animals secrete more virus particles than subclinically sick animals. Thence a more sensitive method detects more subclinicaly ill animals than a less sensitive method. In the present study there was diarrhoea in 36% of all animals, 58% of LA-positive animals and 37% of EM-positive animals were clinically healthy. This also indicates that the LA test is more sensitive than the EM method, or less specific.

Specimens for virus diagnosis should be taken at an early stage of the clinical disease, when the concentration of viruses in stool is greatest. It was not always possible to obtain samples at the beginning of the diarrhoeal period. This might be one reason for the decreased number of positive EM results. In many cases, an initial positive result was obtained with both methods, but in subsequent samples the LA test was more often positive than the EM method. This also indicates that LA is the more sensitive method of the two and probably rather specific too.

With a large number of specimens, the EM method is slow and very laborious compared to the LA test. The equipment needed for EM is sophisticated and experienced staff is needed. On the other hand, the equipment for LA test is simple and can be etablished in every routine laboratory. The test is easy to perform and does not need special skills and is an economical diagnostic method. However, the EM technique is a broader approach and other enteropathogenic viruses than rotavirus may be detected. The LA test is in our opinion suitable for rapid diagnosis of bovine rotavirus as it is more sensitive than EM and also rather specific.

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Sammendrag

Latextest för snabb diagnos av rotavirus hos kalvar. En latex agglutinationstest (LA) jämfördes med direkt elektron mikroskopi (EM) för att påvisa rotavirus infektion hos kalv. Totalt 375 prov från 62 kalvar insamlades. Proven togs när kalvarna var 1, 3, 5, 7, 10 och 20 dagar gamla. Några diarreprov togs också. Allt som allt 45/375 (12%) prov var positiva med LA och 10/375 (2.7%) var positiva i EM. Alla prov som var positiva med EM var också positiva i LA. Av de 62 undersökta kalvarna var 26/62 (42%) positiva med LA och 8/62 (13%) i EM. LA-testen är enligt vår uppfattning lätt att utföra, känsligare än EM-metoden och antagligen relativt specifik för påvisning av rotavirus infektioner.

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