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A COMPARISON OF THREE RAPID DIAGNOSTIC METHODS FOR THE DETECTION OF ROTAVIRUS INFECTION IN CALVES

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ABSTRACT

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Three techniques for the detection of rotavirus in faecal samples from calves with neonatal gastroenteritis were compared. A preliminary study indicated that reverse passive haemagglutination (RPHA) was at least as sensitive as the enzyme-linked immunosorbent assay (ELISA). These two immunoassays were compared with the detection of viral RNA by polyacrylamide gel electrophoresis (PAGE) on 209 field samples. Of the 77 samples in which at least one test gave a positive result, 69 were positive by both RPHA and PAGE, but only 49 were also positive by ELISA, indicating a lower sensitivity for the latter test. The overall agreement between RPHA and PAGE was 96%. The reasons for the discrepancies between the tests are discussed.

INTRODUCTION

Rotavirus plays a major role in the neonatal calf diarrhoea complex (McNulty, 1983), and its importance is reflected in the number of diagnostic tests available. Since infected animals shed very large quantities of virus particles and associated antigenic material in the faeces, electron microscopy (Almeida, 1984) and rapid immunoassays (Reynolds, 1982; Yolken, 1982) have proved particularly appropriate techniques for virus detection.

The most widely exploited immunoassay is the enzyme-linked immunosorbent assay (ELISA), and this has been used at the Central Veterinary Laboratory (CVL) for the routine diagnosis of bovine rotavirus infection since 1982. A different type of test, which identifies rotaviral nucleic acid examined by polyacrylamide gel electrophoresis, has been described recently

(Herring et al., 1982), and preliminary studies at the CVL with this sensitive and specific method indicated that the ELISA was failing to detect some of the positive samples. The present study was initiated to investigate the problem further and to explore the potential of the alternative reverse passive haemagglutination assay (RPHA).

MATERIALS AND METHODS

A polyclonal antibody preparation to a field strain of bovine rotavirus was made by immunising a laying hen with partially purified virus and harvesting the egg yolk antibodies by the method of Jensenius et al. (1981). The yolk proteins were partitioned using dextran sulphate (0.01 mM), and the IgG was precipitated from the aqueous fraction by 40% ammonium sulphate. The IgG was resuspended in, and dialysed against, the appropriate buffer for the subsequent assays.

Murine monoclonal antibody A3M4 (Beards et al., 1984) to the rotavirus group antigen was kindly provided by Dr T.H. Flewett, WHO Collaborating Centre for Reference and Research on Rotavirus, East Birmingham Hospital, Birmingham, England.

The ELISA was developed from the method of Reynolds et al. (1984) using the hen antibody both for antigen capture and, conjugated with horseradish peroxidase, as the detector system. The chromogen was o-phenylene diamine. Specificity of the reaction was monitored by adding the samples to wells coated with antibody lacking any detectable anti-rotavirus activity. This was prepared from eggs from the same hen, collected prior to the rotavirus immunization. Samples were classified as positive if the colour in the test well (coated with virus-specific antibody) was at least 0.2 absorbance units more intense than in the control well.

The RPHA was carried out as described by Cranage et al. (1985) using antibody linked by chromic chloride treatment to chymotrypsin-treated sheep erythrocytes. Faecal suspensions of approximately 1/10 dilution, prepared for the ELISA in phosphate buffered saline (PBS) with 0.05% Tween 20, were further diluted 1/2 in PBS, then mixed with an equal volume of 10% washed sheep erythrocyte suspension. The final faecal dilution was thus 1/40. The mixture was left to settle overnight at 4°C for the absorption of non-specific haemagglutinating activity from the samples. This procedure was adopted for working convenience, and shorter absorptions (e.g. 10 min at 37°C) have also been found satisfactory. Serial doubling dilutions of the supernatant were made in round bottom 96-well microtitration plates. Equal volumes (50 or 30 μ l) of 1% red cells coupled to antibody were added to all wells, and the haemagglutination reaction was read after 1–2 h at room temperature. Non-specific haemagglutination was monitored by the use of red cells coupled to rotavirus-negative antibody. This was either the pre-inoculation egg antibody or, where the monoclonal antibody was used as the detecting antibody, normal mouse IgG.

Polyacrylamide gel electrophoresis (PAGE) was performed essentially as described by Herring et al. (1982). Viral double-stranded RNA was extracted from faeces with phenol-chloroform and run for 16 h on 5% polyacrylamide slab gels, in a voltage gradient of approximately 4 V cm^{-1} . The nucleic acid bands in the gels were subsequently made visible by silver staining. A strong positive control was run on each gel and test samples were recorded as positive if the characteristic 11-segment rotavirus electropherotype could be detected visually.

The samples examined were bovine faeces submitted to the Central Veterinary Laboratory through the Veterinary Investigation Service for the routine diagnosis of neonatal gastroenteritis. In a preliminary study, 20 samples, collected during 1984, were examined by ELISA and RPHA. A second batch consisted of all bovine faeces samples received during a 3-week period in March 1985, amounting to 209 samples after exclusion of those for which insufficient material was available for all three tests. In each case, the person interpreting the test was unaware of the results of the other two tests.

RESULTS

The results of the preliminary comparison on 20 samples between ELISA and RPHA are shown in Table I. RPHA detected all nine ELISA-positive samples, using either monoclonal antibody A3M4 or polyclonal hen antibody. One of the 11 ELISA-negative samples (No. 10) gave a weak positive reaction in the RPHA with both monoclonal and polyclonal antibodies, and the reaction could be blocked with rabbit anti-rotavirus serum. The conclusion that this was a specific reaction was supported by the fact that this sample was from the same outbreak as sample No. 20, which was clearly positive for rotavirus.

The results of the three-way comparison between ELISA, RPHA (both with hen antibody) and PAGE are shown in Table II. It became apparent during the study that non-specific haemagglutination was leading to confusion in interpreting the results of the RPHA with a number of faeces samples. A positive result for RPHA is recorded in Table II only where there was complete agglutination of the anti-rotavirus-coated red cells at least $\geq 1/320$ dilution from the faeces, with no agglutination at the same dilution with the control red cells. The agreement between RPHA and PAGE was particularly good; thus 73 samples were positive by PAGE and 72 by RPHA, although the two methods disagreed on seven samples. ELISA detected only 50 of the 76 samples which were positive by either or both of the other tests. For the 204 samples where a result was obtained from all three tests, the overall agreement between RPHA and PAGE was 96%, RPHA and ELISA 89%, and PAGE and ELISA 87%.

Where sufficient sample was available, discrepant results were further investigated by repeat tests, some using monoclonal antibody A3M4 in the RPHA, and electron microscopy. The ELISA failed to detect 20 samples

TABLE I

Comparison of ELISA and RPHA techniques for detection of rotavirus in calf faeces

Sample No.	ELISA result	RPHA titre (end point dilution of faeces)	
		Hen antibody	Monoclonal antibody
1	-	*	<20
2	-	<20	<20
3	-	<20	<20
4	-	<20	<20
5	-	<20	<20
6	-	*	<20
7	-	<20	<20
8	-	<80	<20
9	-	<20	<20
10	-	20	20
11	-	<20	<20
12	+	320	320
13	+	5120	1280
14	+	1280	640
15	+	1280	640
16	+	5120	2560
17	+	1280	640
18	+	1280	640
19	+	81920	5120
20	+	160	80

*Unable to read.

TABLE II

Comparative efficacy of RPHA, PAGE and ELISA in the detection of rotavirus in bovine faecal samples

Results			No. of samples
ELISA	RPHA	PAGE	
+	+	+	49
-	+	+	20
+	+	-	1
-	-	+	4
-	+	-	2
+	-	-	1
-	-	-	127
-	*	-	5
Total			209

*Unable to read RPHA due to non-specific haemagglutination.

which were positive by the other two tests, and gave a weak reaction in one case which was negative by RPHA and PAGE. A repeat PAGE test on this sample was negative and the ELISA was interpreted as a false-positive. Two samples negative by ELISA and PAGE gave relatively strong positive reactions with both polyclonal and monoclonal antibody in the RPHA. Repeat tests by PAGE were negative but the finding by electron microscopy of very small numbers of rotavirus particles in one of the two samples indicated that the low level of virus in this case was not detectable in the present gel system. Rotavirus particles were not identified in the second sample and the reason for the discrepancy was not clear. One sample positive in both serological tests was consistently negative by PAGE, but insufficient material was available for further analysis.

Four samples were negative by ELISA and RPHA but clearly positive by PAGE. Three of the genome profiles obtained were very weak and repeat tests, in which bands could not be detected, would have been scored as negative. The fourth sample in this group was examined by electron microscopy and contained large numbers of clumped rotavirus particles exhibiting poor morphology. This appearance was consistent with the presence of naturally occurring rotavirus-specific antibody which could have accounted for the negative results in both the RPHA and ELISA tests.

An important, but small, group of samples (negative in PAGE and ELISA tests) could not be read by RPHA due to non-specific agglutination.

DISCUSSION

All three tests described for rotavirus detection can be effectively used at a centralised laboratory where samples can be processed in batches. The simple sandwich ELISA in this study was markedly less sensitive than the RPHA even though it used the same antibody preparation that was used for coating the red cells. The use in RPHA of monoclonal antibody appeared to offer no significant increase in specificity or sensitivity. The sensitivity of ELISA reactions can be enhanced by amplification steps in the detection system, for example by using the biotin avidin system (Yolken et al., 1984) but this would be at the expense of technical and manipulative complexity with corresponding opportunities for error.

PAGE is a sensitive and relatively straightforward technique, which compares well with negative stain electron microscopy (D. Chasey, unpublished observations) often taken as the standard method by which alternative assays are calibrated. Since PAGE depends on the identification of a characteristic and distinctive rotavirus electropherotype, false-positive results are unlikely. This feature, and the non-dependence on antigenicity, also enables the test to detect atypical rotavirus groups which, although uncommon in cattle, may become increasingly important (Chasey and Davies, 1984). Unlike the immunoassays, however, this use of PAGE cannot be extended to other important agents involved in the neonatal diarrhoea syndrome, such as bovine

coronavirus. The present results indicate that PAGE and RPHA have a similar sensitivity for the detection of rotavirus in faecal samples, although neither test appears to detect reliably all positive samples when low levels of virus are present. If used for screening to establish patterns of infection in groups of calves it is likely that either test would give satisfactory results.

RPHA requires a measure of finesse in the coupling of antibody to the erythrocytes. As the coupled cells can be stabilised with glutaraldehyde (Cranage et al., 1983) the coupling could be carried out at a centralised laboratory and cells supplied to smaller diagnostic centres as required. The test protocol is simple and rapid, and can be applied to small numbers of samples or larger batches according to demand. The major limitation of RPHA is the presence of non-specific haemagglutinating factors in a number of bovine faeces samples, although the problem in this study was not as severe as reported in previous work (Bradburne et al., 1979). In most cases non-specific factors can be eliminated by one or more adsorptions of the samples with normal sheep red blood cells. Further improvements may be achieved by the inclusion of serum (rotavirus-antibody-free) or detergents (Tween 20) in the test diluent (Cranage et al., 1985). RPHA has also been applied to bovine enteric coronavirus (Sato et al., 1984) and it should therefore be possible to establish a simple and sensitive testing protocol using RPHA for the two major enteric viral pathogens of young calves.

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