Comparison of electron microscopy, enzyme-linked immunosorbent assay and latex agglutination for the detection of bovine rotavirus in faeces

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ABSTRACT

The performance characteristics of 2 enzyme immunoassays (ELISAs) and 4 latex agglutination assays (LXs) were evaluated for the detection of bovine rotavirus in faecal specimens of young calves with diarrhoea. A total of 26 specimens from calves less than 5 months of age were examined with different commercial assays and compared with electron microscopy (EM) as the gold standard and with polyacrylamide gel electrophoresis (PAGE) for the detection of atypical, non-group A rotaviruses. In the 2nd study, EIA (Dako) and LX (Murex), the assays of choice, were used to analyse 97 further faecal specimens from calves with diarrhoea. The ELISAs proved to be the most sensitive compared with the other tests used. The EM and PAGE are 100 % specific although slightly less sensitive than the commercial assays. The results show that all the commercial assays can accurately detect rotavirus in the stools of calves with gastroenteritis, although the suitability and choice of assay will depend upon the requirements of individual laboratories.

Key words: bovine, calf, diarrhoea, electron microscopy, ELISA, latex agglutination, rotavirus.

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INTRODUCTION

Rotavirus is one of the major agents of morbidity and mortality amongst newborn and young calves with diarrhoea and requires rapid and accurate diagnosis for effective management of calves to limit the spread of infection[12]. Rotavirus disease has been reported to infect up to 80 % of the calves in a herd, with a mortality rate of 30 %[7]; accurate figures are not available for South Africa.

The transmission electron microscope (EM) is a valuable tool for the identification of rotavirus and has remained the reference standard against which other laboratory tests are measured[1]. EM has the added advantage that several different viruses, including those for which no alternative assays are yet available, can also be identified[2]. However, the method of choice in most laboratories has been the enzyme-linked immunosorbent assay (ELISA) because little specialised equipment is required. ELISAs are generally highly sensitive, specific, convenient for testing large numbers of specimens and easy to perform[6]. Many commercial ELISAs for the detection of rotavirus antigen in stools are now available for routine diagnostic use.

Latex agglutination tests for the detection of rotavirus are also available and are an attractive alternative to ELISA, because of their low cost and rapidity[3]. They are simple to operate, require no additional equipment and can be read with the unaided eye, yielding a result within 15–20 minutes of receiving the specimen. However, although the latex agglutination assays are suited to testing small numbers of routine specimens, they are not suitable for screening large numbers of specimens[9].

In this study, we evaluated the performance characteristics of 2 ELISAs (Rotavirus EIA, International Diagnostic Laboratories, Israel, and Rotavirus ELISA, Dakopatts, Denmark) against 4 latex agglutination assays as indicated in Table 1. Negative-stain electron microscopy was used as a reference method for the detection of bovine rotavirus in all faecal specimens.

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MATERIALS AND METHODS

Faecal specimens

Faecal samples were collected from 2 cohorts of young calves with acute diarrhoea. In the 1st study, 26 faecal specimens were collected in 1988 and examined by electron microscopy. These specimens were used to evaluate the 2 EIAs and 4 LXs (Study 1). In the 2nd study, 97 specimens were collected in 1989 from 4 farms situated in the Gauteng, Northern Cape and KwaZulu-Natal Provinces and evaluated by the EIA and LX of choice. Ten to 20 % faecal suspensions were prepared in phosphate-buffered saline (PBS) and stored at –20 °C until further analysis was performed.

Electron microscopy

Transmission electron microscopy was performed with negative staining using 3 % potassium phosphotungstate. Briefly, faecal samples were suspended in PBS and centrifuged for 30 min at 700 g to eliminate macroscopic debris. Successive supernatants were centrifuged at 7000 g for 30 min and then at 48 000 g for 1 h. The final pellet was resuspended in a few drops of Tris buffer (pH 7.4) and negatively stained with 3 % phosphotungstic acid (pH 6.4) on formvar-coated grids. These were examined in a Joel 100 CX transmission EM at a magnification of ×40 000.

Enzyme-linked immunosorbent assay

Two commercially available ELISAs were used in the study. The first, Rotavirus EIA (IDL, Israel) utilises microtitre plates coated with rabbit anti-rotavirus antisera (test strip) and normal rabbit serum to act as a negative control for each specimen tested (control strip). The clarified faecal suspension was added in duplicate to the sample diluent in the wells of the test and control strips and incubated at room temperature for 1 h. After washing, a rabbit anti-rotavirus antibody conjugated to biotin was added to complete the sandwich. Simultaneously, the avidin-peroxidase conjugate was added for the 30 min incubation at room temperature. After a further wash to

Table 1: **Characteristics of the different assay systems.**

Assay	Time to perform	Number of incubations	Solid phase	Capture antigen	Species used
IDL EIA	2.25 h	3	Microstrip	anti-SA11	Rabbit
Dako EIA	4.5 h	5	Microplate	anti-NCDV	Rabbit
Slidex	20 min	1	Latex	anti-RF	Mouse
Pastorex	20 min	1	Latex	anti-SA11	Rabbit
Rotascreen	15 min	1	Latex	anti-Wa	Rabbit
Murex LX	15 min	1	Latex	anti-NCDV	Rabbit

Table 2: **Comparative analysis of different commercial assays in the first study.**

Specimen No.	IDL	Dako	Rotascreen	Murex	Pastorex	Slidex	PAGE	EM
1	+	+	NSA[1]	+	+	NSA	+	+
2	+	+	–	+	–	–	–	–
3	+	+	NSA	+	–	–	–	–
4	–	–	NSA	–	NSA	–	–	–
5	+	+	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–
7	+	+	–	+	–	–	–	–
8	+	+	+	+	NSA	–	+	+
9	–	+	–	+	NSA	+	–	+
10	–	–	+[2]	–	–	–	–	–
11	+	+	NSA	+	–	+	–	+
12	–	–	–	–	–	–	–	–
13	+	+	+	+	+	+	+	+
14	–	–	–	–	–	–	–	–
15	+	+	–	+	–	–	+	+
16	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+
18	–	–	–	–	–	+[2]	–	–
19	–	–	–	–	–	–	–	–
20	+	+	+	+	+	+	+	+
21	+	+	+	+	–	–	–	+
22	–	–	NSA	+	–	+	–	–
23	+	+	+	+	–	–	+	+
24	–	–	–	–	–	–	–	–
25	+	+	+	+	–	–	+	+
26	–	–	–	–	–	–	–	–

[1]NSA = non–specific agglutination in both test and negative control well.
[2]Agglutination in test well only and not in negative control well.

eliminate any unbound avidin-conjugate, the enzyme was added for a 15 min incubation. The degree of enzymatic activity, indicative of the amount of antigen in the stool, was measured colorimetrically by a spectrophotometer at 492 nm.

The 2nd ELISA (Rotavirus ELISA, Dakopatts, Denmark) is more rapid, with an assay time of 30 min. This is achieved by the simultaneous incubation of the faecal extract with a peroxidase conjugated rabbit antibody to bovine rotavirus in microtitre wells coated with rabbit anti-bovine rotavirus and normal rabbit immunoglobulin. After incubation for 15 min at room temperature, the wells are washed and the chromogenic substrate is added and incubated for 3–5 minutes at room temperature. As with the previous ELISA, enzymatic activity can be measured spectrophotometrically.

Latex agglutination

The latex agglutination assays evaluated in this study all utilise a rapid slide test in which latex particles coated with specific antibody react with rotavirus antigen in the faecal extract, resulting in agglutination. The agglutination occurs rapidly and can be read by the unaided eye, obviating the use of expensive equipment. The 4 latex agglutination assays used in this study were: Slidex rota-kit (bioMerieux, France); Pastorex Rotavirus (Diagnostics Pasteur, France); RotaScreen latex test (Mercia Diagnostics, UK); and the Wellcome latex agglutination test (Murex Diagnostics, UK). The characteristics of these commercial assays are illustrated in Table 1.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis of the viral RNA was performed to establish that the rotavirus isolates contained the group A antigen to which the serological reagents of the ELISA and LA tests are directed. The rotavirus RNA genome was extracted by phenol-chloroform treatment and precipitated with cold absolute ethanol as described elsewhere[10]. Electrophoresis of the RNA was performed overnight in 10 % polyacrylamide gels at 100 V[11] and the RNA bands were visualised by silver staining[4].

RESULTS

In the 1st study, 26 faecal specimens from calves with diarrhoea were analysed by the 2 ELISAs and the 4 latex agglutination assays and were evaluated against

Table 3: **Sensitivity, specificity and diagnostic accuracy of the different tests.**

Assay	Specificity (TN/TN+FP)[a]	Sensitivity (TP/TP+FN)[a]	Predictive value	
			Positive	Negative
EM	10/10 (100)	12/16 (75)	12/12 (100)	10/14 (71)
PAGE	10/10 (100)	9/16 (56)	9/9 (100)	10/17 (59)
Dako EIA	10/10 (100)	16/16 (100)	15/15 (100)	10/11 (91)
IDL EIA	10/10 (100)	15/16 (94)	16/16 (100)	10/10 (100)
Murex	9/11 (82)	15/16 (94)	15/15 (94)	9/10 (90)
Pastorex	9/10 (90)	5/16 (31)	5/5 (100)	9/18 (50)
Slidex	8/11 (73)	7/16 (44)	6/8 (75)	8/17 (47)
Rotascreen	7/11 (64)	9/16 (56)	8/9 (89)	7/16 (44)

[a]TN = true negative results; TP = true positives; FN = false negatives; FP = false positives.

Table 4: **The specificity and sensitivity of the assay systems for the detection of bovine rotavirus.**

Assay	Specificity (%)	Sensitivity (%)	Diagnostic accuracy (%)
Dako EIA	69/69 (100)	28/28 (100)	97/97 (100)
Murex LX	69/74 (93)	23/28 (82)	92/97 (95)
EM	69/69 (100)	22/28 (79)	91/97 (94)

EM and PAGE (Table 2). Of the 2 ELISAs, the Dakopatt Rotavirus ELISA detected an additional rotavirus-positive specimen that was confirmed by EM, although very few particles were seen. Both ELISAs were, in general, more sensitive and specific than the latex agglutination assays, although the Murex latex agglutination assay showed comparable results (Table 3).

Amongst the latex agglutination assays, all but the Murex test gave results showing non-specific agglutination, *i.e.* agglutination in both the test and the negative control wells, which interfered with the diagnostic efficiency. It is noteworthy that the Murex test uses antisera raised to a bovine rotavirus (NCDV) as does the Slidex (RF), which showed no non-specific reactions and only 1 non-specific agglutination reaction, respectively. In addition, the Murex test was shown to be more sensitive than the other latex agglutination assays, detecting more rotavirus-positive specimens and was comparable in sensitivity to the ELISAs (Tables 2, 3).

Because the immunoassays are able to detect only group A rotaviruses, we included PAGE to determine the serogroup of any potential EM positive/ELISA negative specimens that might have been non-group A rotaviruses. No rotavirus strains with the characteristic RNA profile of non-group A rotaviruses were detected in this study.

In the 2nd study, the Dako ELISA and the Murex latex agglutination assay were utilised to evaluate their test performances for the detection of rotavirus in 97 faecal specimens. The results of the study are shown in Table 4.

DISCUSSION

There is a need for rapid and simple diagnostic techniques for the viral agents of gastroenteritis. As rotavirus is an important agent associated with gastroenteritis in humans and domestic animals[7], various methods for the detection of rotaviral antigen in stool specimens have been developed. Transmission electron microscopy and solid phase immunoassays have been shown to be suitable for the detection of small quantities of rotavirus in stool samples[5].

Transmission electron microscopy has been used as the gold standard against which most other diagnostic tests have been evaluated[2]. The electron microscope is valuable because it gives a rapid and definitive result and it also has the advantage of being able to detect other viruses present in the stools that may have a causal role in producing gastroenteritis[1]. However, the electron microscope is not suitable for the smaller laboratory because of the prohibitive cost of the equipment, the need for an experienced and qualified technician, and the fact that it is not suited for the daily examination of many specimens.

On the other hand, enzyme immunoassay systems are widely used because they provide for the rapid detection of rotavirus antigen in a relatively short period[6]. Commercial ELISA kits are available for the routine diagnostic laboratory that may have to screen large numbers of specimens. These commercial kits provide all the necessary reagents, are easy to use and are sensitive and specific for the detection of rotavirus antigen in faeces. In this study, the 2 ELISA systems performed very well recording high specificity and sensitivity (Table 3). The major drawbacks of the system are that it is not cost-effective for testing single specimens and that it cannot detect non-group A rotaviruses.

The latex agglutination assays have a number of advantages, including the ease and rapidity of the test and the low cost per test[3,9]. No specialised equipment is needed and the results can usually be read by the unaided eye, which makes it suitable for use in rural practice.

Two specimens were positive with EM and negative with the other systems used to screen the field specimens. These could be atypical rotaviruses that are not detectable by the immunoassays developed to date. PAGE is the only system able to differentiate between group A and atypical rotaviruses at present[8]. However, the PAGE results were negative for these 2 specimens and could not confirm that they are atypical strains.

All the diagnostic procedures analysed in this study have shown that they are accurate and reliable for the detection of bovine rotavirus in faeces. Although the ELISA test has been the most widely used assay for the detection of rotavirus in calf diarrhoea[1], the latex agglutination assay is a rapid and accurate alternative that could be widely used in southern Africa.

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Book review — Boekresensie

Comprehensive reports on technical items presented to the International Committee or to Regional Commissions 1996

1996. Office International des Épizooties, 345 pp. Price: FrF 150, US$ 30. ISBN 92 9044 424 X.

This collection of reports includes chapters on the following subjects of veterinary interest: genetic biotechnology; aspects of health control in the movement of animal product, including the Office International des Épizooties recommendations and procedures for complying with the requirements set by the World Trade Organization (WTO), the role of risk analysis in regionalisation, and the effect of infrastructures on surveillance; the role of carrier animals in the transmission of foot-and-mouth disease; updates on the transmissible spongiform encephalopathies; and surveillance control of fish diseases.

The report on the application of biotechnology for the genetic improvement of livestock provides a useful summary of the available technologies. The author classifies these as reproductive biotechnology, livestock genomics and marker-assisted selection, and transgenics. While techniques such as artificial insemination and embryo transfer are widely used in livestock improvement in both developed and developing countries, the application of the more sophisticated techniques for genetic manipulation is more limited at present. It is evident that certain aspects of genetic selection, for example for resistance to particular diseases or to select for or against other desirable heritable traits, could be very usefully applied at all levels of livestock production.

Several chapters relate to the development of strategies for safe animal and animal product movement under the conditions created by WTO and the General Agreement of Tariffs and Trade (GATT), and the role of the OIE in preventing spread of animal diseases in the absence of restrictive controls. In these reports the Agreement of Sanitary and Phytosanitary Measures under the WTO is clarified, and aspects such as regionalisation and risk assessment and management are addressed. A chapter on the effect of infrastructures on surveillance and monitoring systems emphasises the importance of adequate veterinary structures for accurate surveillance and reporting, and the need for adequate systems for disseminating information.

Two reports provide useful updates on scrapie and bovine spongiform encephalopathy (BSE), respectively. The chapter on scrapie concentrates on the situation in certain countries in the Americas, but provides useful information on recent research on transmission and on diagnostic methods of choice and surveillance. A report on the epidemiology, pathogenesis and research aspects of transmissible spongiform encephalopathies in animals and humans correlates the information contained in reports from 14 European countries, with particular reference to BSE and Creuzfeldt-Jakob disease (CJD).

A chapter on the role of carrier animals in the transmission of foot-and-mouth disease indicates that shedding of virus by persistently infected (carrier) animals rarely causes outbreaks, and highlights the need for further research in this field.

In the report entitled 'Surveillance and control of marine fish diseases', many of the fish considered are usually classed as freshwater products, *e.g.* trout and eels. The term 'fish farming' is also used by the author in its broadest sense, to include both farming with captive-bred stock and growing-out of wild-caught juveniles (a sort of aquatic feedlot). The author suggests methods based on disease control in mammals to be applied to both surveillance and disease control in fish. Disease control in fish-farming operations is often of concern to aquatic environmentalists, since most treatments result in large quantities of chemical used for medication entering public water sources. It is encouraging to note that Norway has managed to develop vaccines to control the major salmonid diseases, resulting in a drop in sales of antimicrobial drugs for salmonid farming from 50 000 kg in 1987 to less than 5000 kg in 1995. An article of 11 pages can only touch briefly on such a complex subject, where advances in both management technique and veterinary knowledge are rapid, but further details can be obtained from the excellent list of references, of which no less than 60 of the 66 papers date from 1990 onwards. It is, however, surprising that no mention is made of disease in marine aquarium fish.

This collection of reports contains a considerable amount of information that is of value to veterinarians in a number of fields, but most importantly to those involved in import and export of animal products who need to understand the WTO and GATT approaches and to explain them to their clients.

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