THE NEUTRALISATION OF THE INDIRECT IMMUNOFLUORESCENCE TEST AND THE IMMUNODIFFUSION TEST : TWO DETECTION METHODS FOR NEONATAL CALF DIARRHOEA VIRUSES. A COMPARATIVE STUDY WITH ELISA AND ELECTRON MICROSCOPY

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ABSTRACT

A comparative study of the neutralisation of the indirect immunofluorescence (NIIF) test, the immunodiffusion (ID) tes, enzyme-linked immunosorbent assay (ELISA) and electron microscopy (EM) for the detection of rotaviruses in calf faeces was done at the Nationaal Instituut voor Diergeneeskundig Onderzoek (NIDO) in Belgium and the Centraal Diergeneeskundig Instituut (CDI) in The Netherlands. A total of 93 faecal samples was examined. Sixty-two per cent was positive for rotavirus by EM, 59 % with the NIIF test, 58 % with the ID test and 57 % with ELISA. All samples negative by EM were also negative in the NIIF and the ID test but three of these samples were found positive in the ELISA.

Forty-three faecal samples were tested for the presence of bovine coronavirus. Eleven samples were scored positive by EM of which ten were positive in the NIIF test and five in ELISA. Two other samples were found positive by ELISA only.

INTRODUCTION

In recent years several new methods have been developed for the detection of enteric viruses in faecal material, f.e. enzyme-linked immunosorbent assay (ELISA) (1, 3, 4), the neutralisation of the indirect immunofluorescence (NIIF) test (6) and an immunodiffusion (ID) test (5). To compare the results of these methods with each other and with those of electron microscopy (EM), a comparative study was undertaken in which the Nationaal Instituut voor Diergeneeskundig Onderzoek (NIDO) in Belgium and the Centraal Diergeneeskundig Instituut (CDI) in The Netherlands participated.

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MATERIAL AND METHODS

Faecal samples

The faecal samples used in this study originated from diarrhoeic as well as healthy calves. The majority of the samples had been tested previously in one of the participating laboratories, i.e. they had been frozen twice at -20° C (before and after testing) before they were sent to the other laboratory.

A total of 93 samples was examined for the presence of rotaviruses in the NIIF test, the ID test and by EM in the NIDO, and by EM and ELISA in the CDI. In addition, 43 samples were examined for the presence of bovine coronaviruses in the NIIF test and by EM in the NIDO and by ELISA in the CDI.

Virus detection methods

The ELISA's for the detection of rotavirus and bovine coronaviruses in faecal samples have been described (1, 3). Further details can be found in the rotavirus manual of the Central Veterinary Institute, The Netherlands, in these proceedings.

The NIIF test and the ID test have also been described (6, 5); laboratory manuals of both tests are included in these proceedings.

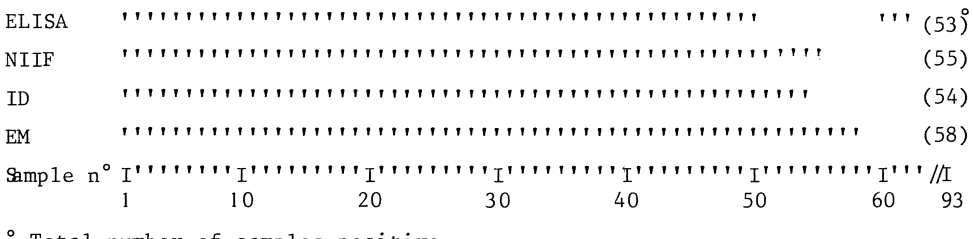
Electron microscopy was performed as follows. In the NIDO faecal material was diluted 2:3 in PBS, mixed with an ultraturrax, and centrifuged at 3,000 rpm for 15 min. The supernatant was applied to carbon-coated grids and strained with a solution of 2 % uranyl acetate. The grids were examined for 15 min using a Philips TEM201 electron microscope. In the CDI faecal extracts were applied to carbon-coated grids and stained with a solution of 2 % PTA adjusted to pH 6.2 with KOH. Ten squares of a 400-mesh grid were examined for the presence of rotavirus particles using a Jeol 100C electron microscope at a magnification of 50,000.

Each faecal sample was tested in two institutes; a sample is recorded rotavirus-positive if at least one of the examinations yielded positive results.

RESULTS

The results obtained with the different tests for the detection of rotavirus are shown on fig.1.

Fig. 1. Comparison of different tests for the detection of rotavirus in faeces of calves

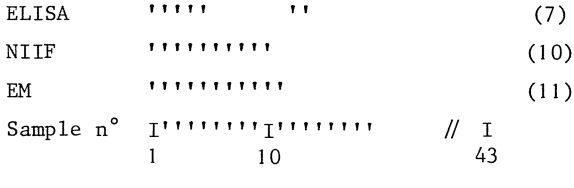


° Total number of samples positive

Fifty-eight out of 93 faecal samples were scored positive by EM, 55 in the NIIF test, 54 in the ID test and 53 by ELISA. Rotavirus antigens were detected by all four tests in 50 samples and by three tests in four other samples. With ELISA three samples were found positive that were negative in the other tests.

Fig.2 shows the results of the different tests for the detection of bovine coronaviruses. Eleven out of 43 samples were scored positive by EM; ten in the NIIF test and seven by ELISA. Five samples were positive in all three tests, ten samples in two samples were scored positive by ELISA only.

Fig.2 Comparison of different tests for the detection of bovine coronaviruses in faeces of calves.



CONCLUSIONS

The results of the four tests used in this study for the detection of rotavirus in faecal samples corresponded rather well (Fig.1). The unexpectedly high EM score is undoubtedly influenced by the fact that each sample was tested in two different laboratories and that the time spent on each sample was unusually long. Furthermore, rotavirus-antibody complexes present in faecal samples may prevent a positive diagnosis in the ELISA, the NIIF and the ID test, whereas they are easily detected by EM (2). The specificity of the NIIF and the ID test is demonstrated by the fact that all samples found positive in these two tests were also scored positive by EM.

Whether the three faecal samples that reacted positively in the ELISA but not in the other tests are indeed positive remains an open question, despite the fact that they passed the specificity check, i.e. the blocking test.

Since all tests employed in this study for the detection of rotaviruses in faecal samples of calves appear to be of comparable sensitivity and specificity, the choice for a particular laboratory may be based on the future use and the local circumstances. If an electron microscope is available and limited numbers of samples have to be examined, EM appears to be the method of choice. The ID test is probably the most simple test of the four employed here; neither expensive equipment nor tissue culture facilities are required. The NIIF test has the advantage that it does not require mono-specific antisera. This may be of particular significance for laboratories that do not have the disposal of gnotobiotic or SPF calves. The ELISA has the usual advantages of this type of assay, i.e. the large numbers of samples that can be handled and the speed with which the results become available. If necessary the ELISA can be completed within the same day, whereas the ID test takes 24 h and the NIIF test 36 h.

The results of the different tests used for the detection of bovine coronaviruses (Fig.2) indicate that the NIIF test is nearly as sensitive as (extensive) EM and slightly more sensitive than ELISA. However, as only a limited number of samples tested were positive, definite conclusions await futher work. In addition, the fact that the faecal samples had been frozen and thawed at least twice before they could be tested in the other laboratory, may have had more influence on the detection of bovine coronaviruses than on that of rotaviruses.

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