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DETECTION OF PORCINE ROTAVIRUS BY EM, ELISA AND CIET*

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ASKAA, JON and BUCHARDT BLOCH: *Detection of porcine rotavirus by EM, ELISA and CIET.* Acta vet. scand. 1981, 22, 32—38. — Feces samples from swine-herds with severe problems of neonatal diarrhoea, 3 weeks scours or early weaning diarrhoea were examined for porcine rotavirus using three techniques: EM, ELISA and CIET. Infection with rotavirus was found in about one third of the feces samples, representing more than half of the examined swine-herds. EM and ELISA revealed nearly all the samples found to be positive, while CIET as used here detected only half of them.

porcine rotavirus; EM; CIET; ELISA; diarrhoea.

Rotavirus has been detected in pig feces since 1975 (*Rodger et al.* 1975) and considered to be the cause of, or to be a contributory agent for, certain forms of neonatal diarrhoea, 3 weeks scours and early weaning diarrhoea (*Rodger et al., Lecce et al.* 1976, *McNulty et al.* 1976, *Woode et al.* 1976, *Chasey & Lucas* 1977, *Bohl et al.* 1978, *Tzipori & Williams* 1978, *Debouck & Pensaert* 1979). Further the diarrhoea complex has been reproduced experimentally by inoculation of field isolates and of in vitro cultured virus on colostrum-deprived piglets (*Lecce et al., McNulty et al., Woode et al., Theil et al.* 1977, 1978, *Bohl et al., Debouck & Pensaert*).

Infections with rotaviruses are generally diagnosed by electron microscopy (EM), but also immunofluorescence staining of intestinal smears, of cryostat sections of small intestine, or of inoculated cell cultures have been used as well as complement fixation test, counter-current immunoelectrophoresis test (CIET) and enzyme-linked immunosorbent assay (ELISA) techniques

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(*Woode et al., Chasey & Lucas, Saif et al.* 1977, *Theil et al., Ellens & de Leeuw* 1977, *Ellens et al.* 1978, *Bachmann* 1979, *Debouck & Pensaert*).

In the present work, the prevalence of porcine rotavirus infection in Danish swine-herds with diarrhoea problems was examined by EM, CIET, and an ELISA, originally developed for diagnostic work on human rotavirus infection (*Grauballe et al.* 1981).

MATERIALS AND METHODS

Feces samples were collected from swine-herds in various parts of the country. The only criterium for selection of the materials was the existence of a severe continuous diarrhoea problem in the swine-herd. Within each herd, between 1 and 5 feces samples were taken, each from a different litter. Samples representing 110 litters in 42 swine-herds were examined.

Feces were diluted with Eagles essential medium (Gibco, U.K.) to make a 20—30 % solution. The samples were clarified by centrifugation at 3000 × g for 20 min. The supernatants were used for ELISA or stored at —40°C until further processing. Suspensions for EM and CIET were made by layering 2.5 ml supernatant on top of 5 ml of 45 % sucrose and centrifugating at 200.000 × g at 15°C for 3 h in a Sorval T 865 angel rotor. The drained pellets were resuspended in 1 ml of distilled water.

EM

A drop of the resuspended pellet was placed on 400 mesh formvar-carbon coated grids and stained with 1 % uranyl acetate (pH 4.0). The coated grids were pretreated with a drop of a Bacitracin solution containing 10 µg/ml in order to improve the spreading of the materials (*Gregory & Pirie* 1972). The grids were examined using a JEOL JEM 100B electron microscope at an acceleration voltage of 80 kV. On each grid at least 3 squares were examined.

CIET

The test was performed on 10 × 10 cm glassplates with 1.5 mm thick gels of 1 % agarose (Litex HSA, Denmark), The buffer (pH 8.6) consisted of Tris (hydroxymethyl) aminoethane 4.81 g, diemal acid 2.05 g, sodium diemal 8.13 g, and distilled water to make 1 l. Pairs of wells, 3 mm in diameter and with a center-to-center distance of 10 mm, were punched in the gel. The resuspended pellet was applied to the cathodic well and a hyperimmune rabbit serum prepared against purified porcine rotavirus was applied to the anodic well, both in volumes of 10 µl. Electrophoresis was performed overnight at

2V/cm and the plates were stained with Coomassie Brilliant Blue®. The hyperimmune serum was prepared by inoculation of a purified rotavirus preparation. A field isolate of porcine rotavirus was used as source of virus for purification. Feces were treated with trichlorotrifluorothane, clarified and layered on a 17.5 % sucrose freeze gradient in a buffer (pH 7.6) consisting of 0.040 mol/l sodium phosphate and 0.2 mol/l sodium chloride. The gradient was fractioned after ultracentrifugation at 100.000 × g at 10°C for 7 min in a Sorval TV 865 rotor. To ensure minimum contamination the virus containing fractions were examined by EM before inoculation in the rabbit.

ELISA

The test was performed according to *Grauballe et al.* (1981), using the Ig-fraction of rabbit antibodies with specificity to human rotavirus as catching antibodies, and the same antibodies coupled to horseradish peroxidase as detecting antibodies. Briefly, every second well of polystyrene microtest plates (NUNC®, Roskilde, Denmark, code 2-62162) was coated with catching antibodies (anti-human rotavirus rabbit IgG, Dako-immunoglobulins Ltd., Copenhagen, Denmark, code B 218) in dilution 1:10.000 with 0.05 mol/l carbonate buffer (pH 9.6), while the other wells were coated with normal rabbit IgG (Dako-immunoglobulins, code X 904) in the same dilution and the same buffer. The plates were washed and feces samples were added. For each sample, 2 wells coated with anti-human rotavirus IgG, and 2 wells coated with normal rabbit IgG were used. After incubation overnight the plates were washed and detecting antibodies, i.e. peroxidase conjugated anti-human rotavirus rabbit IgG (Dako-immunoglobulins, code P 219) in dilution 1:250 was added. The enzyme substrate was 1.2 phenylenediamine-dihydrochloride (Sigma). The absorbance at 492 nm was measured at a Spectronic 21 (Bauch & Lomb) spectrophotometer. A feces sample was considered to be positive for content of rotavirus antigens, if the E_{492} value was above 0.1 and at least 3 times that of the negative control (the microwells coated with normal rabbit IgG).

RESULTS

Porcine rotavirus was detected in 37 of the 110 feces samples, representing 23 of 42 swine-herds with diarrhoea problems. The anamnestic information indicates either neonatal diarrhoea, 3 weeks scours or early weaning diarrhoea as a problem in the examined herds. Seven of the virus isolates were connected with neonatal diarrhoea, 22 with 3 weeks scours, and 3 with early weaning diarrhoea. In 5 cases the age of the litter was not given.

The results of the examinations appear from Table 1. The EM and the ELISA techniques revealed 35 and 33 rotavirus positive feces samples respectively of the 37 samples in which virus was demonstrated by either of the 3 methods used. In the CIET,

Table 1. Feces samples collected from piglets with diarrhoea classified by content of porcine rotavirus as detected by electron microscopy (EM), enzyme linked immunosorbent assay (ELISA) and counter-current immunoelectrophoresis test (CIET).

Type of reaction	Number of samples
EM+, ELISA+, CIET+	18
EM+, ELISA+, CIET—	13
EM+, ELISA—, CIET—	4
EM—, ELISA+, CIET—	2
EM—, ELISA—, CIET—	73
Total	110

18 of the samples were rotavirus positive. In 4 of the samples only EM confirmed the rotavirus diagnosis, and in these cases only a few rotavirus particles were detected. By ELISA rotavirus antigen was detected in 33 feces samples, and of these 31 were confirmed by EM. The 2 remaining feces samples represent herds in which samples from other litters were found positive.

The samples were often seen in the electron microscope to

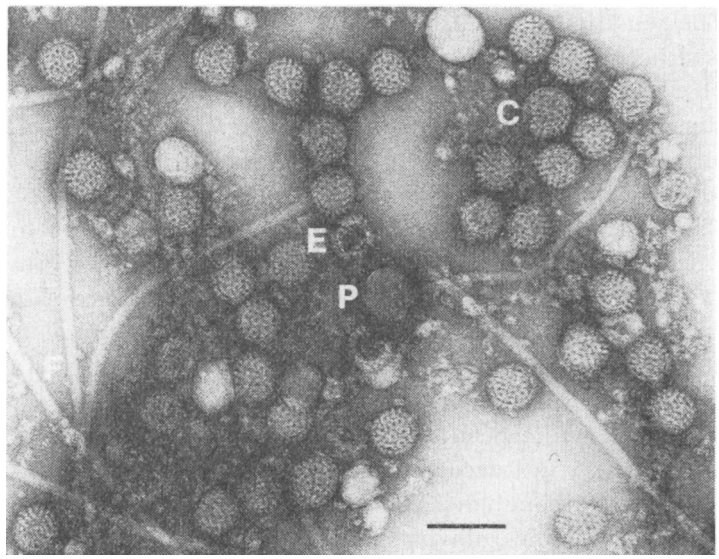

Figure 1. Porcine rotavirus in a partially purified feces sample. The majority of the virions lack the outer capsid layer that gives the complete virions a circular outline (C). Also a few empty capsid shells are present (E). Besides the virions a phage (P) and bacterial flagellas (F) can be seen. The bar represents 100 nm.

contain bacterial residues, e.g. flagellas and cell walls, and also a number of different phages could be present, together with possible rotavirus (Fig. 1).

DISCUSSION

In addition to an isolate described by *Grauballe et al.* (1979), infection with porcine rotavirus in Denmark has been described by *Askaa et al.* (1980). The detection of porcine rotavirus in ⅓ of the feces samples and in more than half of the examined swine-herds shows a wide spread and frequent occurrence of rotavirus infection in Danish swine-herds. These findings correspond to descriptions from other countries. Thus *Bohl et al.* (1978) examined 10 swine-herds with rotavirus associated diarrhoea problems especially correlated to 3 weeks scours. *Bachmann et al.* (1979) found rotavirus in 2 of 47 feces samples from 2 to 15 days old piglets with diarrhoea, and *Corthier et al.* (1980) found rotavirus in 24 % of 42 examined farms, without mentioning the age of the piglets and the type of diarrhoea. Meanwhile our material contains a particularly high proportion among 3—4 weeks old piglets of rotavirus associated diarrhoea.

The sensitivity of EM and ELISA seems equal, but the CIET as used here revealed only about half of the samples found positive by the other 2 methods. These findings agree with results obtained for rotavirus infections of other species (*Spence et al.* 1975, *Middleton et al.* 1976, *Grauballe et al.* 1977, *Ellens et al.* 1978).

The possibility of using antibodies with specificity to human rotavirus in ELISA for diagnostic detection of porcine rotavirus exists because of a common antigen for rotaviruses (*Kapikian et al.* 1976, *Grauballe et al.* 1979). Also *Bachmann et al.* (1979) used a heterologous conjugate for detection of porcine rotavirus infection. It will, however, be of importance to compare the use of an ELISA with specific antibodies against porcine rotavirus with the ELISA test according to *Grauballe et al.* (1981). Meanwhile it may be concluded that the latter can be used in routine detection of porcine rotavirus infection.

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SAMMENDRAG

Påvisning af porcin rotavirus ved hjælp af EM, ELISA og CIET.

Fæcesprøver fra svinebesætninger med længere varende problemer med neonatal, tre ugers eller tidlig fravænnings diarré blev undersøgt for tilstedeværelse af porcin rotavirus ved hjælp af elektronmikroskopi (EM), "enzyme linked immunosorbent assay" (ELISA), og modstrømselektroforese (CIET). Rotavirus blev påvist i omkring en trediedel af fæcesprøverne og i mere end halvdelen af de undersøgte svinebesætninger. I næsten alle rotavirus-positive prøver fandtes virus ved hjælp af EM og ELISA, mens det kun var muligt at påvise virus i halvdelen af alle rotavirus-positive prøver ved hjælp af CIET.

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