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Solid phase immune electron microscopy for diagnosis of transmissible gastroenteritis in pigs

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A serological trapping technique is described for detecting transmissible gastroenteritis (TGE) virus in faeces. The technique involves the coating of electron microscope grids with protein A and specific TGE virus antiserum. Optimal conditions for performing this solid phase immune electron microscopy technique were a concentration 250 μg ml⁻¹ of protein A; 1:100 diluted rabbit anti-TGE virus hyperimmune serum for coating the grids and overnight incubation with virus samples. The possibility of detecting coronavirus in crude faeces was highly improved with solid phase immune electron microscopy, compared with conventional negative staining electron microscopy, by specific trapping of virus and prevention of adsorption of contaminants. The sensitivity of the method was evaluated by coded investigation of a dilution series of stock virus mixed with different pools of faeces. The improvement of virus detection in faeces by solid phase immune electron microscopy, compared with standard electron microscopy was at least 100-fold. Faecal shedding of coronavirus by pigs infected with virulent and attenuated strains of TGE virus was studied. Virus detection in faeces by a standard electron microscopy technique was not practical, since the virus was obscured by a large quantity of debris in the faeces. By using solid phase immune electron microscopy, however, the aspect of the specimens on the grids improved so much that, in addition to more common immunofluorescence, the technique might be useful as a diagnostic test for TGE.

Virus was detected in daily faecal samples from one or two days after experimental infection with virulent TGE virus, until death in five out of eight animals. Unlike immunofluorescence, solid phase immune electron microscopy may be used for diagnosis in living animals.

The inability of wild strains of transmissible gastroenteritis (TGE) virus to grow efficiently in conventional cell-culture systems has resulted in the use of diagnostic methods such as immunofluorescence on tissue sections of the small intestine and electron microscopy on intestinal contents. Immune electron microscopy can greatly increase the sensitivity of diagnostic electron microscopy. Conventional immune electron microscopy is based on the observation in the electron microscope of clumps of virions specifically formed with homologous antibodies (Saif et al 1977).

Derrick (1973) described a new immune electron microscopic technique in which grids were coated with antibodies and used for specific trapping of plant viruses. Shulka and Gough (1979) introduced a modification in which grids were precoated with protein A before coating with specific antiserum. Precoating of the grids with protein A increases the efficiency of trapping by the absorbed antibodies, since the immunoglobulin molecules are attached to the grid by their Fc regions and have their antigen binding sites preferentially exposed. This technique, called solid phase immune electron microscopy (SPIEM), has been applied successfully in the detection of rotavirus in human stools (Nicolaieff et al 1980, Svensson et al 1983).

Materials and methods

Chemicals

The following abbreviations for chemicals and solutions were used: PBS, phosphate buffer (0.01 M) in saline (0.14 M), pH 7.3; PBS-BSA, 0.1 percent (w/v) solution of bovine serum albumin (Sigma) in PBS; PBS-Tween, PBS containing 0.05 percent (w/v) Tween 80 (Merck); PBS-FBS, PBS containing 10 percent fetal bovine serum (Gibco); protein A, a lyophilised derivative from Staphylococcus aureus (Pharmacia). Protein A was reconstituted with PBS to a concentration of 250 μg ml⁻¹. This preparation was stored in a -20°C freezer.

Virus strains and preparation of virus stocks

Virulent Miller and attenuated Purdue strains of TGE virus were kindly provided by Professor Pensaert, University of Ghent, Belgium. The history of these strains was described by Bohl et al (1972). The Miller strain was used to infect germfree pigs orally. Pigs were killed shortly after the onset of diarrhoea (about 24 hours after exposure), and intestinal
contents were washed out with PBS. The contents were sonicated and centrifuged for 10 minutes at 1000 g; the supernatant was filtered through a 5 μm filter. The virus suspension (denoted stock A) was used for experimental infections and as a standard for optimisation of test conditions in a SPIEM assay.

TGE/Purdue virus was propagated in SK6 cells (Kasza et al 1972). Cells were cultured in Eagle's basal medium supplemented with a protein hydrolysate mixture and 5 per cent FBS (Barteling 1972). Confluent monolayers were infected with approximately 10,000 plaque forming units (PFU) per cell and incubated in medium without serum. Virus was harvested by two cycles of freezing and thawing of infected cells in culture medium, followed by 10 minutes centrifugation in culture medium at 1000 g. The supernatant had an infectivity titre of 24 × 10⁶ PFU ml⁻¹ in SK6 cell monolayers. The virus suspension (denoted stock B) was used for experimental infections and as a standard in SPIEM.

Partly purified TGE/Purdue virus was obtained by harvesting culture medium of infected SK6 cells at an early stage of cytopathological effect. Virus was concentrated with PEG 6000 and purified by centrifugation over a discontinuous gradient: 28 ml 36 per cent (w/v) and 4 ml 60 per cent (w/v) sucrose in a tube of the SW27 rotor of a Beckman LS-65 ultracentrifuge run at 90,000 g for 180 minutes. This partly purified virus had an infectivity titre of 2.5 × 10⁶ PFU ml⁻¹. This virus suspension (denoted stock C) was used as a standard in SPIEM. Highly purified virus for immunisation of rabbits was obtained by centrifugation of PEG concentrated virus on a continuous glycerol-tartrate gradient (45 to 0 per cent w/v glycerol and 0 to 50 per cent w/v di-K-tartrate in 10 mM Tris-HCl, pH 7.5) for 18 hours at 90,000 g (Obyeski et al 1974). Virus was located at a density of 1.24 g cm⁻³.

Antiserum and conjugate

A rabbit was immunised intramuscularly with 120 μg purified TGE/Purdue virus in mineral oil adjuvant (Herbert 1965). The animal was boosted intramuscularly 47 days later with 900 μg virus in mineral oil adjuvant and bled 21 days afterwards. The neutralising antibody titre of the antiserum was 40,000 expressed as the reciprocal of serum dilution resulting in a neutralisation of 100 TCID₅₀ of TGE/Purdue virus. Conjugate for immunofluorescence was prepared from immune serum obtained from a specific pathogen free pig which had been exposed orally to the Purdue strain of TGE virus and had been given intramuscularly three subsequent doses of partly purified virus in mineral oil adjuvant over a period of four months. The serum, collected a week after the last inoculation, contained a neutralising antibody titre of 1:20,000. Conjugation of the gammaglobulin fraction of this serum with fluorescein isothiocyanate was performed as described by Ressang (1971).

Electron microscopy

Solid phase immune electron microscopy, standard procedure. All incubations were performed at room temperature. 400 mesh nickel grids were used, giving 400 squares in a grid. Freshly prepared grids, supported with a collodion film and reinforced with carbon, were floated for five minutes on a drop of solution of protein A in PBS (250 μg ml⁻¹). The grids were then washed on three drops of PBS and drained on filter paper. Protein A coated grids were transferred for 10 minutes incubation on a drop of rabbit anti-TGE virus serum, diluted 1:100 in PBS. The grids were washed again on three drops of PBS-BSA and incubated overnight on a drop of virus sample; they were washed a final time by placing them on a series of six drops of PBS and drained on filter paper. Fixation was carried out during one minute with 1 per cent glutaraldehyde in PBS-Tween. The grids were rinsed with one drop of PBS-Tween, stained for 10 seconds with 2 per cent phosphotungstic acid pH 6.8 and examined in a Philips EM-300 electron microscope at an accelerating voltage of 80 kV and an instrument magnification of 20,000. Mean numbers of virus particles per grid square were estimated from at least two squares chosen from a part of the grid where contrast by staining was superior.

Scores were assigned as follows: +, mean particle number per square less than one; 2+, one to five particles per square; 3+, six to 50 particles per square; 4+, 51 to 500 particles per square; and 5+, more than 500 particles per square. In experiments for testing optimal conditions for a SPIEM test, virus particles were counted in five or 10 randomly chosen squares, and the means and standard deviations were calculated.

Faecal extracts were prepared by diluting the samples 1:5 in PBS and shaking with glass beads. For SPIEM, a protein A-antiserum coated grid was floated on a drop of a 1:2 dilution in PBS-FBS of the faecal extract.

Standard EM

For conventional negative staining electron microscopy (standard EM), faecal extracts were clarified by low speed centrifugation, and grids with a collodion support were incubated for 10 minutes with a drop of supernatant. Additionally, supernatant was centrifuged at 10,000 g for five minutes, and the pellet was resuspended in a drop of demineralised water and tested for virus particles occurring in complexes of cellular debris. Fixation was performed with 1 per
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FIG 1: Effect of different concentrations of protein A (0, 2.5, 25 and 250 µg ml⁻¹) and different dilutions of antiserum (1:100, 1:500 and 1:2500) used for coating grids on trapping of coronavirus particles from a TGE/Purdue virus suspension, partly purified from medium of SK6 cells (a) and from a faecal suspension of a pig infected with TGE/Miller virus (b). Incubation of grids with virus samples was overnight.

cent glutaraldehyde in PBS-Tween and staining with 2 per cent phosphotungstic acid. Since contamination of faecal specimens by debris allows no reliable estimation of numbers of virus particles in standard EM, grids were examined (10 minutes screening time) for the presence of coronavirus particles and scored only positive or negative.

Experimental pigs

Caesarean-derived, colostrum-deprived pigs of three litters were used for experimental infections. Two litters were raised under germfree conditions in an isolator; a third litter was reared under specific pathogen free conditions. Animals were fed sterile condensed milk, and creep feed was offered from 10 days old. Creep feed contained 19 per cent protein, was free from antibiotics and was sterilised by γ-radiation. Specific pathogen free pigs in the third experimental infection were maintained on condensed milk until animals were infected at an age of 19 days. A sterile electrolyte solution containing 2 per cent (w/v) glucose, 0.35 per cent (w/v) sodium chloride, 0.25 per cent (w/v) sodium bicarbonate and 0.15 per cent (w/v) potassium chloride was given from the onset of diarrhoea.

Experimental infections

Experiment 1. Two 12-day-old germfree pigs were infected orally with 1 ml of a bacteria-free faecal suspension containing the virulent Miller strain of TGE virus (stock A). Faeces were collected twice a day until animals were necropsied at eight days after infection. Samples were processed for examination by SPIEM, and as described before, a score for the number of virus particles was assigned.

Experiment 2. Three 20-day-old germfree pigs were infected orally with 1 ml of a faecal suspension with virulent TGE/Miller virus (stock A). Three other pigs from the same litter were kept in a separate isolator and were infected with 1 ml of attenuated TGE/Purdue virus (stock B). Faeces were collected from infected pigs twice a day, until the animals died or virus could no longer be detected in their faeces by SPIEM.

Experiment 3. Three 19-day-old specific pathogen free pigs were infected orally with 1 ml of a faecal suspension with virulent TGE/Miller virus (stock A). Faeces were collected twice a day until the third or fourth day when animals died spontaneously, or in consequence of diarrhoea, were in extremis and were slaughtered for post mortem examination.

Histopathology and immunofluorescence

Specimens from sites at approximately 5 (duodenum), 20, 35, 50, 65, 80 and 95 (ileum) per cent along the length of the small intestine and from the caecum were obtained, fixed in 10 per cent neutral buffered formalin and processed for paraffin tissue sections according to conventional methods. Sections were stained with haematoxylin and eosin and examined for histological lesions of TGE. Sections of
identical sites of the intestine of uninfected control pigs were prepared and the length of villi from normal and infected pigs were compared. Cryostat sections were obtained from the same parts of the intestine and tested for the presence of TGE virus antigen in epithelial cells by an immunofluorescence test (Pensaert et al 1968).

Results

Effect of different concentrations of protein A and dilution of antiserum on trapping of virus particles

Grids were arranged in three groups: four grids in each group were coated with 0, 2.5, 25 and 250 µg ml⁻¹ of protein A in PBS, respectively. Each group was then coated with different antiserum dilutions: 1:100, 1:500 and 1:2500. The trapping efficiency of the grids was examined after overnight incubation with a 1:500 dilution of partly purified TGE/Purdue virus (stock C) or a 1:4 dilution of a faeces suspension containing TGE/Miller virus (stock A). After fixation and staining, trapping efficiency of each grid was evaluated by counting virus particles in five squares.

An increase in the number of virus particles bound to protein A and antiserum-treated grids, compared with those treated with antiserum alone, was generally observed (Fig 1). Highest virus trapping efficiency was achieved with a protein A concentration of 250 µg ml⁻¹ and a 1:100 dilution of antiserum. Under optimal conditions, 20 times more virus particles were trapped from a TGE/Purdue virus suspension (Fig 1a) and six times more from a TGE/Miller virus suspension (Fig 1b), compared with grids coated with antiserum alone. A concentration of 250 µg ml⁻¹ of protein A and a 1:100 dilution of antiserum were chosen for a standard SPIEM test.

Effect of period of incubation on trapping of virus particles

Grids coated with 250 µg ml⁻¹ protein A and a 1:100 dilution of the antiserum were used. The number of trapped virus particles increased with time of incubation: with a 1:10 dilution of a cell-culture harvest of TGE/Purdue virus (stock B), the number was 16 times higher after 20 hours incubation than after one hour incubation (Fig 2a); with a 1:2 dilution of faecal suspension of TGE/Miller virus (stock A), the number was eight times higher after an incubation of 20 hours than after one hour's incubation (Fig 2b). Overnight incubation was chosen for a standard SPIEM test.

Comparison of sensitivity of SPIEM and standard EM

The number of virus particles trapped on a grid by SPIEM and standard EM were compared. A 1:10 dilution of cell-culture harvest of TGE/Purdue virus (stock B) was incubated overnight with a protein A antiserum-coated grid in a standard SPIEM assay. For standard EM, grids were incubated with the same virus stock diluted 1:4. Numbers of virus particles were counted in five squares in both tests. Seventeen times as many virus particles were detected in virus stock B by SPIEM, compared with standard EM. The aspect of both by EM is shown in Fig 3c and d.

A major effect of SPIEM might be expected from specific trapping of virus particles out of a faeces suspension with much contamination of faecal contents. The improvement of the aspect of an EM grid from a crude faecal sample by SPIEM, compared with standard EM is illustrated in Fig 3a and b.

To evaluate the sensitivity of SPIEM for coronavirus detection in faeces, the following experiment was carried out: a series of 10-fold dilutions of a faecal suspension of TGE/Miller virus (stock A) was prepared. The virus dilutions were diluted 1:10 with PBS (F in Fig 4) or with different pools of faecal suspensions (A to E) obtained from virus negative pigs, and screened for coronavirus by coded investigation in a SPIEM test. SPIEM scores of virus suspensions in faeces were plotted against dilutions of the virus (Fig 4). The undiluted stock of TGE/Miller virus contained 2213 particles per square in a standard SPIEM test (mean of four independent tests). A significant correlation was found between the SPIEM score of different samples and virus dilution. The Kendall's coefficient of rank correlation was 0.87 which is significant at the P = 0.001 level (Conover 1971). The SPIEM score did not deviate significantly from the expected number of virus particles in the samples (sign test P > 0.05). Only those samples containing the highest concentrations of virus particles were positive by standard EM.

For a comparison of the sensitivity of SPIEM and standard EM, 33 faecal samples were screened: 20
FIG 3: (A) Aspect of faeces containing TGE/Miller virus as seen by standard EM. vp Virus particle. (B) The same sample prepared by SPIEM. (C) Aspect of cell culture harvest of TGE/Purdue virus as seen by standard EM. (D) The same sample prepared by SPIEM
samples were dilutions of stock TGE/Miller virus in faeces (mentioned earlier); 13 samples were from pigs infected experimentally with TGE/Miller virus and were selected for their high concentration of virus particles in a SPIEM test. Out of 16 samples with a SPIEM score of 4+ and 5+ (over 50 particles per square), 11 were positive by standard EM (Table 1). By standard EM, more than one particle was seldom found per square in these samples. Virus was detected in supernatant after low speed centrifugation of the faecal suspension, in sediment after high speed centrifugation or in both. Out of 17 samples with a SPIEM score of three plusses or less, only one sample was positive by standard EM.

In this experiment, faecal specimens containing at least 50 particles per square in a SPIEM test gave a 70 per cent chance of finding the virus by screening several squares using standard EM technique. In a dilution series of a standard virus suspension in different pools of faeces, only three out of five 1:20 diluted samples were positive by standard EM, while all five 1:2000 diluted samples and three out of five 1:20,000 diluted samples were positive by SPIEM. In conclusion, the improvement of virus detection in faeces by SPIEM is at least 100-fold.

Reproducibility of SPIEM

A 1:10 dilution of cell culture harvest of TGE/Purdue virus (stock B) was tested as a reference in 14 independent SPIEM tests, performed according to the standard procedure. Numbers of virus particles in two squares were counted in each test, and mean and standard deviations were calculated. A 1:4 dilution of a faecal suspension with TGE/Miller virus (stock A) was tested similarly in nine different tests. Mean numbers of 406 (so = 109) and 438 (so = 119) particles per square were found in the diluted stocks of TGE/Purdue and TGE/Miller virus. Coefficients of variation were 34 and 27 per cent, respectively.

Distribution of virus particles on a grid

A 1:15 dilution of faecal suspension with TGE/Miller virus (stock A) was tested by SPIEM according to standard procedures, and numbers of virus particles were counted in 55 grid squares chosen at random. The same virus suspension was diluted 1:2 for overnight incubation with a grid coated with 1:100 diluted antiserum without precoating with protein A, and virus particles were counted in 55 squares.

The mean number of virus particles in 55 squares of
**Experiment 1.** Diarrhoea was observed in the two infected pigs from one day after infection until the eighth day when both animals were killed for post mortem examination. Both pigs had villous atrophy along the whole length of their intestine, and fluorescing epithelial cells were found in most parts of their small intestines. Excretion of coronavirus in faeces was detected by SPIEM from one to five days after infection (Fig 5, pig 2).

**Experiment 2.** Infection with the virulent Miller strain of TGE virus caused diarrhoea from one day after infection until animals died spontaneously (Fig 5, pigs 11 and 22), or until the second day after infection in the only animal that recovered (pig 17). Autolysis hampered judgement of histopathological lesions in pigs that had died of the TGE virus infection at four and seven days after infection. Nevertheless, fluorescence was found in the caudal part of the small intestine of both animals. Coronavirus particles were detected by SPIEM in faeces from one day after infection until animals died, or until nine days after infection in the only pig that recovered from the infection. Virus excretion reached levels of more than 500 particles per square in some samples.

Diarrhoea was less severe in pigs infected with the attenuated Purdue strain and was observed on the fifth and sixth day after infection in pigs 12 and 20. No animals died. Excretion of Purdue virus was observed from the fourth day after infection in two pigs and from the first day in one pig and lasted until the seventh or ninth day (Fig 6). Concentrations of virus particles per square were detected by SPIEM in faeces from one day after infection until animals died, or until nine days after infection in the only pig that recovered from the infection. Viral excretion reached levels of more than 500 particles per square in some samples.

Diarrhoea was less severe in pigs infected with the attenuated Purdue strain and was observed on the fifth and sixth day after infection in pigs 12 and 20. No animals died. Excretion of Purdue virus was observed from the fourth day after infection in two pigs and from the first day in one pig and lasted until the seventh or ninth day (Fig 6).

A protein A antiserum-coated grid after incubation with a faecal suspension of TGE/Miller virus was 198 (SD = 60). The Kolmogorov-Smirnov test for goodness of fit did not show a significant deviation from a normal distribution (P = 0.8) (Conover 1971). A mean number of 18 particles per square was found on a grid coated with antiserum alone (SD = 33). A significant deviation from a normal distribution was found in this case, indicating an asymmetrical distribution of the virus over the surface of the grid (Kolmogorov-Smirnov test, P < 0.001). This result was mainly due to 20 empty squares containing no particles.

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**FIG 5:** Faecal shedding of coronavirus detected by SPIEM in germfree and specific pathogen free pigs infected orally with virulent TGE/Miller virus. Pigs 11, 17 and 22: germfree animals, infected at 20 days old. Diarrhoea was observed in pigs 11 and 22 from day 1 after infection until death and in pig 17 on day 1 and 2 after infection. Pig 2: germfree animal, infected at 12 days old; diarrhoea from day 1 to 8 after infection. Pig 23: specific pathogen free animal, infected at 19 days old; diarrhoea from day 1 to 4 after infection.

**FIG 6:** Virus excretion in daily faecal samples of three germfree pigs that were infected orally with TGE/Purdue virus (attenuated strain) at 20 days old. Diarrhoea was observed on days 5 and 6 after infection in pigs 12 and 20.
virus particles were generally lower in pigs infected with attenuated Purdue virus than in pigs infected with virulent Miller virus.

**Experiment 3.** Diarrhoea was observed in all three pigs from one to three or four days after infection. Villous atrophy was found over the whole length of the small intestine of both animals that were examined post mortem. Positive fluorescence was found in the latter half of the small intestine. Excretion of coronavirus was detected by SPIEM from the first or second day until animals died (Fig 5, pig 23).

In total, five out of eight pigs infected with the virulent TGE/Miller virus died spontaneously or were killed in extremis, and only one pig survived the infection. Two pigs were slaughtered for post mortem examination on the eighth day when animals still had diarrhoea. At autopsy, virus could be detected in intestinal villi by immunofluorescence in six animals (Table 2); in five animals, faeces were still positive by SPIEM. Virus shedding in faeces could be detected by SPIEM in all animals from the first or second day until animals died (Fig 5, pig 23).

**Discussion**

Negative contrast transmission electron microscopy is suggested by Saif and Bohl (1986) as a method for diagnosing TGE. Application of this method, however, is not often reported in the literature. In the present study, coronavirus particles were rarely found by standard EM in faeces of experimentally infected pigs. Sensitivity of EM for detection of virus may be increased by immune EM of which there are several modifications. By SPIEM, small numbers of virus particles can be visualised more easily by specific trapping of virus. Moreover, a layer of serum proteins prevents the adsorption of crude materials and provides fairly clean preparations. SPIEM has further advantages which make it practical as a diagnostic test: (a) a reduction of time needed to screen one specimen; (b) a more homogeneous distribution of virus particles on the support film, thereby a higher incidence of positive scores; (c) the ability to distinguish, through the specificity of immunological trapping, infections with TGE virus and porcine epidemic diarrhoea virus, a coronavirus which is antigenically unrelated to TGE virus (Pensaert and De Bouck 1978).

Precoating the grids with protein A improved their virus-trapping capacity in comparison with grids coated only with antiserum. The highest number of virions were seen on grids coated with 250 μg ml⁻¹ of protein A and 1:100 diluted antiserum; these conditions were chosen for further work. The present study indicates that increasing the concentration of antiserum does not always improve the virus-trapping efficiency of the grids. Optimal concentration of protein A and antiserum may depend on the antibody titre and need to be determined for each new system.

Svensson et al (1983) reported that SPIEM was 30 times more sensitive than direct EM for detecting rotavirus in faeces. In the present study, 17 times more coronavirus particles were trapped from a relatively pure cell culture harvest of TGE virus by SPIEM than were trapped by standard EM. Virus suspensions prepared from infected cell cultures show relatively little contamination with cellular debris, and the number of virus particles on grids prepared for standard EM can easily be counted. Standard grids of faecal suspensions are largely obscured by debris preventing a reliable estimation of numbers of virus particles. A major improvement of SPIEM is its high efficiency of virus trapping out of heavily contaminated specimens such as faeces; virus could be detected in at least 100-fold higher dilutions of stock virus in faeces, compared with standard EM. Generally, specimens that were just positive by standard EM contained more than 50 virus particles per square in a SPIEM assay.

Immunofluorescence on cryostat sections of the small intestine is the common technique for diagnosing TGE. This technique performed well in proving an infection in the present study; immunofluorescence was still positive in large parts of the small intestines of two animals that were necropsied at eight days after infection. Fresh tissue is required, however, for proper judgement of immunofluorescence, and such tissue is not always available from field outbreaks. In

### Table 2: Results of IFT and virus detection in faeces by SPIEM after experimental infection with the virulent TGE/Miller virus in eight pigs

| Experiment | Pig number | Virus shedding in faeces as detected by SPIEM (days after infection) | IFT Day after infection |
|------------|------------|---------------------------------------------------------------|------------------------|
| 1          | 2          | 1,2,3,4,5                                                     | Pos 8                  |
| 2          | 4          | 1,2,3,4,5                                                     | Pos 8                  |
| 3          | 11         | 1,2,3,4,5,6,7                                                 | Pos 7                  |
|            | 17         | 1,2,3,4,5,6,7,8,9                                             | NA                     |
| 23         |            | 1,2,3,4                                                      | Pos 4                  |
| 24         |            | 2,3,4                                                        | Pos 4                  |
| 32         |            | 1,2,3                                                        | NT                     |

* Animals died spontaneously or killed in extremis
NA Does not apply
NT Not tested

table
such cases, a SPIEM test might be useful in confirming a diagnosis. Moreover, since the present study indicates that the virus can be detected in daily faecal samples of diarrhoeic animals, SPIEM can confirm a diagnosis on living animals. In standard EM, the virus on the grids is obscured by faecal debris, and therefore, the technique does not meet the requirements of a diagnostic test.

Faecal shedding of virulent TGE virus has been reported by Pensaert et al. (1970) over a two week period in animals surviving an infection. In this study, in vivo titration in pigs was used to prove the presence of virus in faeces, since the virulent strain of the virus must adapt in order to grow in cell cultures. SPIEM might be an alternative technique for investigating daily virus excretion in faeces. In the present study, immunofluorescence detected virus replication in epithelial cells of the small intestines of two pigs that were necropsied on the eighth day after infection; this finding is in accordance with results of Pensaert et al. (1970) who detected fluorescence until seven days after inoculation. Restriction of infection by the attenuated Purdue strain of TGE virus to the caudal portion of the small intestine may explain why this strain does not cause diarrhoea as severe as that observed with the virulent Miller virus (Frederick et al. 1976). The results obtained in this study indicate less faecal shedding of virus over a shorter period by pigs infected with the attenuated Purdue virus; this finding agrees with reports which detected limited replication of the virus in the small intestine.

In conclusion, the diagnosis of TGE is usually confirmed by immunofluorescence on cryostat sections of the small intestine. Since autolysis occurs rapidly in the intestines of dead animals, slaughtering sick animals to obtain fresh tissue material is preferred. EM of faeces by SPIEM appears to be a valuable additional technique in confirming a TGE diagnosis and makes diagnosis on living animals possible.

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