INFECTION AND IMMUNITY, Sept. 1976, p. 804-810

Vol. 14, No. 3
Printed in U.S.A.

Morphological and Antigenic Relationships Between Viruses (Rotaviruses) from Acute Gastroenteritis of Children, Calves, Piglets, Mice, and Foals

G. N. WOODE,* JANICE C. BRIDGER, JOANNA M. JONES, T. H. FLEWETT, A. S. BRYDEN, HEATHER A. DAVIES, AND G. B. B. WHITE

Institute for Research on Animal Diseases, Compton, Newbury, Berkshire, England; Regional Virus Laboratory, East Birmingham Hospital, Birmingham, B9 5ST, England; and Public Health Laboratory, Fazackerley Hospital, Liverpool, L9 7AL, England*

Received for publication 29 March 1976

The reovirus-like particles present in the feces of young pigs and foals with acute enteritis and the virus causing epizootic diarrhea of infant mice were found to be indistinguishable morphologically from each other, from the South African SA.11 and "O" viruses, and from the rotaviruses of children and calves. The inner capsid layer of each of these viruses reacted serologically with sera of children, calves, mice, piglets, and foals convalescent from infection with their respective rotaviruses. These sera reacted by immunofluorescence with human, bovine, porcine, and murine rotaviruses, SA.11, and "O" viruses in tissue cultures and with human, bovine, porcine, and murine viral antigens by complement fixation and gel diffusion. However, the antisera differed in their ability to react serologically with the outer capsid layer of the viruses investigated and in their ability to neutralize tissue culture-adapted calf virus. These two tests may demonstrate strain or host specificity among rotaviruses. Since the porcine, murine, and equine viruses are closely related serologically to and are morphologically identical to the human and bovine viruses, they should be included in the group of viruses for which the term "rotavirus" has been suggested. All known members of this proposed group of viruses share a common antigen, probably situated within the inner capsid layer; thus, any one of the viruses may be used for the preparation of antigen or antibody for diagnostic tests, and this will aid in the diagnosis of virus infection in those species from which a rotavirus has not been cultured.

Electron microscopy has revealed virus particles of a characteristic morphology in feces from young children and calves suffering from acute infectious diarrhea (2, 10-12, 17, 19, 21, 27). The bovine and human enteric viruses were shown to be closely related serologically and indistinguishable from each other morphologically (14), and they differed in morphology and antigenicity from the rather similar orbiviruses (5). It was considered that these enteric viruses constituted a new group and that they should be called "rotaviruses" (14). This conclusion was supported by Lecatsas (20), who further suggested that the two viruses isolated from a rectal swab from a monkey (SA.11) and from intestinal washings of sheep and calves (the "O" virus) should be included in the group (9). The antigenic similarity between the human and bovine viruses was confirmed (18, 19). Nucleic acid analysis has shown similarities between the genomes of bovine rotavirus, reovirus 3, and bluetongue virus, and an analysis of polypeptides has emphasized the similarity between the bovine and human rotaviruses (22a, 23).

We have shown that the virus of calf diarrhea is transmissible to piglets and that sera from several pig herds neutralize the calf virus in cell culture. The human rotavirus will infect pigs also and induce an antibody response (7a). Recently, similar viruses, which are morphologically similar to the bovine and human viruses, have been isolated from several outbreaks of diarrhea in pigs (26, 27a) and found in feces of foals with diarrhea (13). Epizootic diarrhea of infant mice (EDIM) is also known to be caused by a virus, and in electron micrographs of negatively stained particles the virus was similar to the human and bovine rotaviruses (17, 22; G. A. Hall, J. C. Bridger, R. L. Chandler, and G. N. Woode, Vet. Pathol., in press). Flattened tubules with hexagonally packed

subunits in association with virus particles were observed in human, bovine, and murine fecàl preparations (15, 17).

We present evidence here that the infant mice, piglet, and foal diarrhea viruses are similar in morphology and antigenicity to the children and calf rotaviruses.

MATERIALS AND METHODS

Animals. Gnotobiotic calves and pigs were produced and reared as described previously (25; M. J. Dennis, D. C. Davis, and M. N. Hoare, Br. Vet. J., in press). Mice obtained from a colony free from the virus of EDIM were reared conventionally.

Cell cultures. Monolayers of primary calf kidney (CK) and primary pig kidney (PK) cells and the IBRS2 line of PK cells were prepared as described previously (27). The growth medium consisted of Earle salt solution, 0.5% (wt/vol) lactalbumin hydrolysate (Nutritional Biochemicals Corp.), 0.1% (wt/vol) galactose, penicillin (100 IU/ml), streptomycin (100 μg/ml), amphotericin B (5 μg/ml) (Fungizone; E. R. Squibb and Sons, Inc.), and 10% fetal calf serum (Flow Laboratories Ltd.). For maintenance of the cells, 2% fetal calf serum was used.

Primary mouse kidney (MuK) cell cultures were prepared by trypsinizing kidneys from young adult mice from the EDIM-free colony. Primary human embryo kidney cell cultures (HEK) from human embryos 10 to 14 weeks of age were prepared in a similar manner. African green monkey kidney (AGMK) cells were purchased from Flow Laboratories Ltd. as a primary monolayer and then trypsinized to give secondary cultures. These cell cultures and LLC MK_2 (Flow Laboratories Ltd.) cells were grown in Eagle minimal essential medium containing 10% fetal calf serum, which was reduced to 2% for maintenance.

Viruses. Bacterium-free filtrates were prepared from diarrheic feces of naturally infected calves and pigs and fed to gnotobiotic calves and pigs at ages ranging between 6 h and 5 weeks (27, 27a). Isolates of the pig virus were obtained from weaner pigs with diarrhea on two separate farms. Three different filtrates of virus-containing feces from children with acute diarrhea were fed orally to pigs on three separate occasions and passaged twice in gnotobiotic piglets (7a). A filtrate of virus-containing feces from a foal was fed to two gnotobiotic piglets. Virus was harvested from the intestinal contents or feces when diarrhea commenced or daily for 14 days after infection.

The murine virus (EDIM) was kindly supplied by D. A. J. Tyrrell and fed to infant mice, 3 to 5 days old, by placing a small drop of suspension in the mouth or on the snout. After 4 to 5 days the mice were killed and the virus was harvested by homogenizing the large and small intestines together in phosphate-buffered saline (PBS), pH 7.2 (1 ml/intestine). This suspension was centrifuged at 2,000 × *g* for 15 min to remove all debris and bacteria; the supernatant, which was found to be bacteriologically sterile, was used as seed virus.

SA.11 and O viruses were kindly supplied by H. H. Malherbe as infected cell culture fluids.

Virus culture. Filtrates of feces or intestinal contents, from the experimentally infected pigs, calves, and mice diluted threefold in PBS (pH 7.3), were inoculated into flying cover slip cultures of CK and PK cells to give a final dilution of 1:30. After 24 to 72 h the cultures were washed in PBS, air-dried, and fixed in acetone. For demonstration of virus-infected cells, the fixed cultures were first treated with human, bovine, or porcine antiserum to the relevant virus and then counterstained with fluorescein-conjugated rabbit antiserum to human, bovine, or porcine gamma globulin (Nordic Pharmaceuticals and Diagnostics, Tilburg and London) (27).

Since EDIM virus did not replicate in MuK cells by conventional techniques, cells in suspension were inoculated. A primary monolayer of MuK cells was trypsinized and resuspended in fresh growth medium to give 3×10^5 cells/ml; EDIM virus suspension was added (0.1 ml/ml of cell suspension), and the virus-cell suspension was allowed to settle and was spread on flying cover slips. After incubation at 37°C for 24 h the cells were fixed in acetone for 5 min at room temperature and stained by the immunofluorescent technique with a human serum. Filtrates of human and murine virus were inoculated into HEK cell cultures (1).

Secondary AGMK monolayers were inoculated with SA.11 and O viruses and after 2 to 3 days of incubation at 37°C were fixed and stained as for EDIM virus; in both cases free virus was present in the culture medium.

Virus morphology. Human, bovine, porcine, murine, and equine viruses were prepared for electron microscopy from feces or intestinal contents by differential centrifugation, followed by centrifugation through sucrose when necessary (14). The murine virus was also prepared for electron microscopy by making impression preparations from the lining of the duodenum and ileum of infected mice on glass slides. These were then rubbed up in a drop of distilled water and mounted on grids for negative staining (15). Plain Formvar and also Formvar membranes reinforced with carbon were used.

To compare the diameters of the virus particles, micrographs of human, bovine, porcine, murine, and equine viruses were taken in succession, using the same specimen holder and always adjusting the magnification control in the same way. At Birmingham, a Philips EM200 microscope was used; this was calibrated with the aid of beef liver catalase and crocidolite crystals. Alternatively, at Compton, diameters were measured from micrographs made with a Philips EM300 microscope, using catalase crystals as an internal standard (27a).

Serology. Convalescent and randomly selected sera from humans, pigs, calves, and mice and antisera prepared from gnotobiotic calves and pigs experimentally infected with human, bovine, porcine, and equine rotaviruses were tested for the presence of antibodies by the following methods.

(i) NT. Neutralization test (NT) titers of sera were determined by reacting twofold serum dilutions with 100 mean tissue culture doses of the calf

cell culture-adapted virus for 1 h at 37°C; 0.2 ml of each virus-serum mixture was inoculated into each of two tubes of CK cells on flying cover slips, with 1.8 ml of maintenance medium (6). The cultures were fixed after 6 days and examined by immunofluorescence for evidence of virus infection.

(ii) FA tests. Immunofluorescent antibody (FA) tests were conducted on CK, PK, $IBRS_2$, MuK, HEK, and LLC MK_2 cell cultures infected with fecal and intestinal filtrates of bovine, porcine, and murine viruses or with the cell culture-adapted bovine virus. These cultures were air-dried, fixed in acetone, and stained by the indirect immunofluorescent method, using human, bovine, porcine, and murine sera and the corresponding fluorescein-conjugated anti-gamma globulin sera (27).

(iii) GD studies. Gel diffusion (GD) studies of sera were undertaken by means of a micro-Ouchterlony-Elek technique. Lines of precipitation between antigens and antisera were developed by double diffusion in an agarose gel consisting of 0.9% agarose (BDH) dissolved in a buffer composed of 0.1 M sodium chloride, 0.01 M tris(hydroxymethyl)aminomethane and 0.001 M ethylenediaminetetraacetic acid.

Volumes (3 ml) of the above gel were dispensed to lidded immunodiffusion trays (Hyland), and when the medium had set a template was employed to cut a rosette of five peripheral wells around a single central well. According to need, wells were either 2 or 4 mm in diameter and were spaced 2 mm apart. In general, antigens (concentrated from feces as for the complement fixation [CF] test) were placed in the larger wells and antisera were placed in the smaller wells. The trays were incubated overnight at 37°C in a humid atmosphere and were read with the aid of a lens and dark-field viewing box.

(iv) IEM studies. The two methods used for immunoelectron microscope (IEM) studies were as described previously (14). For human and some calf fecal preparations 1 drop of serum (either undiluted or 20% [vol/vol] or 10% [vol/vol], according to the stage of disease) was mixed with 5 drops of fecal suspension, prepared by resuspending the deposited virus in PBS after centrifugation at 30,000 rpm for 30 min. After 2 h at room temperature or 4°C overnight, the mixture was made up to 5 ml and centrifuged at 30,000 rpm for 30 min. The deposits were resuspended and negatively stained with 2% potassium phosphotungstate at pH 5.5. This technique was modified for some calf fecal samples that required further clarification: 0.02-ml volumes of serial antiserum dilutions were added to equal volumes of virus suspensions prepared by differential centrifugation and sucrose gradient centrifugation (24). After incubation at 37°C for 2 h, a drop of the mixture was negatively stained and examined in the electron microscope.

(v) CF tests. Antigens for use in CF tests were suspensions of virus concentrated from clarified human, calf, piglet, and foal feces and mouse intestines by ultracentrifugation. These antigens were titrated by the "chessboard" technique against convalescent human, calf, and mouse sera (16). For the test, 2% sensitized sheep erythrocytes and 4 mean hemolytic doses of guinea pig complement were used. The tests were set up in microtiter plates, using 0.025-ml volumes of reagents. Fixation was overnight at 4°C.

(vi) Control reagents. For all serological tests control reagents included gnotobiotic calf antiserum to reovirus 3, gnotobiotic pig antiserum to transmissible gastroenteritis (corona) virus of pigs, preinoculation gnotobiotic calf and pig sera, and virus-free feces obtained from milk-fed gnotobiotic calves and pigs. Gut suspensions from uninoculated infant mice and sera from uninfected adult mice were also used. These control reagents consistently gave negative results.

RESULTS

Animal infection. The bovine, porcine, and murine viruses consistently caused diarrhea in calves, pigs, and mice, respectively, after incubation periods of 24 to 92 h for calves and pigs and 48 to 96 h for mice. All animals recovered after an illness lasting 4 to 6 days, and virus was recovered from the feces for several days after the onset of diarrhea. Calves and pigs aged between 2 days and 5 weeks were susceptible to infection. The bovine virus also caused diarrhea in piglets, but the human and equine viruses did not, although the human virus was found in the piglet feces 5 to 7 days postinoculation in pass 1 and on day 3 in passes 2 and 3 (7a); the equine virus was recovered in piglets 4 days postinoculation. Two calves fed orally with the human virus and one calf fed orally with the porcine virus neither excreted virus nor developed antibody.

Virus morphology. In negatively stained preparations it was not possible to distinguish by size and shape virus particles from the feces of children, calves, infant mice, piglets, and foals suffering from diarrhea, the SA.11, O, and calf virus from infected tissue cultures, or those found in the feces of piglets fed the human, bovine, porcine, equine, and foal viruses (Fig. 1). Particles both with and without the outer capsid layer were found for each animal species; the diameters of the particles ranged from 50 to 69 nm, depending on the presence or absence of the outer capsid layer. Diameters measured from micrographs of viruses mounted on carbon-Formvar membranes were consistently 10% larger than when they were mounted on plain carbon membranes. The outer layer of the mouse virus was easily lost, so that "complete" particles were usually very scanty in preparations made from clarified emulsions of infected mouse gut. Complete particles were, however, plentiful in impression preparations from mouse intestinal mucosa. The outer layer of capsids of EDIM virus could be stabilized by

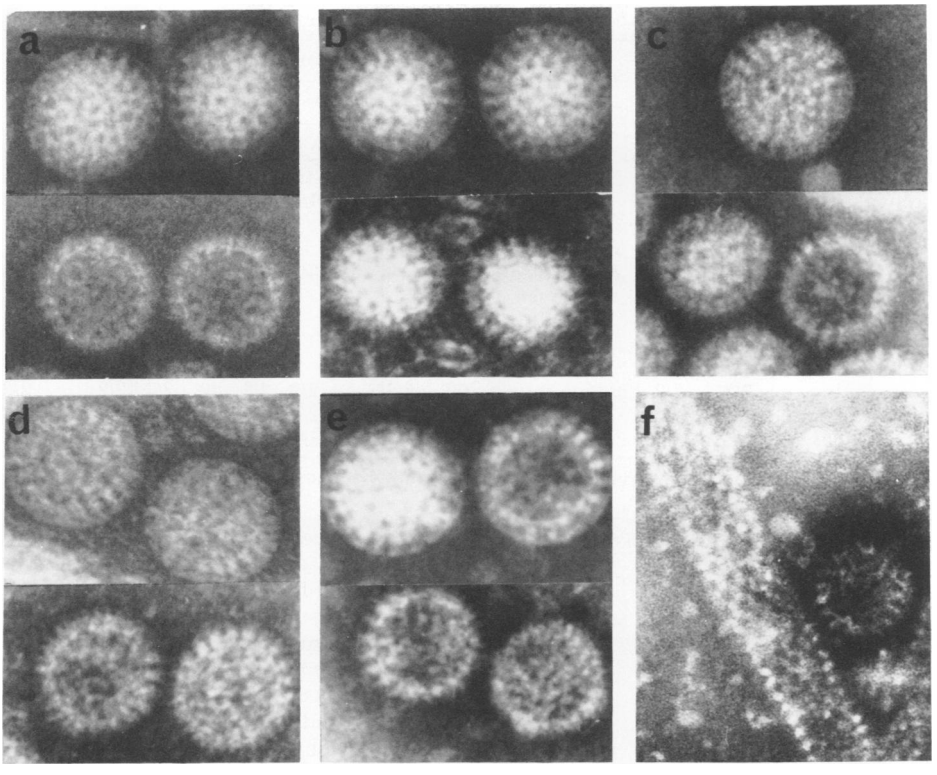

FIG. 1. *Negatively stained rotavirus particles from a (a) man, (b) calf, (c) mouse, (d) pig, and (e) foal. Intact smooth particle above and incomplete rough particle below. ×296,000. (f) shows tubule from foal preparation. × 222,000*

resuspending the impression smears or by homogenizing the intestines in 4% formaldehyde; thus fixed, they appeared to retain serological reactivity as determined by electron microscopy. Complete particles of all these rotavirus types showed a characteristic "spokelike" arrangement of inner capsomeres surmounted by an outer layer, giving the particles a smooth, clearly defined rim. When this outer layer was lost, the inner capsomeres projecting from the surface made the particles appear "rough" around the edge. Both with and without the outer capsid layer, particles often displayed a "ring-shaped" arrangement of the capsomeres, as described by Borden et al. (5) as a characteristic of the orbiviruses. These rings could be caused to appear and disappear by tilting the virus in an eucentric goniometer stage. Flattened tubules with hexagonally packed subunits were seen in preparations of human, bovine, murine, and equine rotaviruses.

Serology. The serological cross-reactions between the rotaviruses are summarized in Tables 1 and 2. Antibodies to the bovine rotavirus were also demonstrated in randomly collected sera of rabbits, guinea pigs, goats, and sheep by NT, FA, or GD.

Although all human and animal antisera to the rotaviruses reacted with the bovine rotavirus by IEM, FA, CF, and GD, this activity did not correlate with NT or agglutination of smooth versus rough virus particles by IEM. To examine this phenomenon further, the titers of the antisera by NT and FA were compared. The NT titer and FA titer of the standard gnotobiotic antiserum to the bovine virus were 640 and 160 to 320, respectively. A similar relationship was shown with three other bovine antisera. Similarly, those human and pig antisera with NT titers of 20 to 160 had FA titers equal to or two- to fourfold lower than the NT titer. In contrast, human and pig antisera that did not neutralize the bovine virus, including sera from pigs convalescent from infection by the human and porcine viruses, had titers of 40 to 1,280 by the FA test to the bovine tissue culture-adapted virus. These results show that the NT and FA antigens are distinct.

To determine whether pigs infected with the porcine virus and initially lacking neutralizing

TABLE 1. *Serological cross-reactions between the human, bovine, porcine, murine, and equine rotaviruses*

Antiserum	Viral antigen (no. of positive/no. assayed)														
	Human		Bovine				Porcine			Murine			Equine		
	IEM[a]	CF	NT	FA	IEM	CF	FA	CF	IEM	FA	CF	IEM	FA	CF	IEM
Human random		11/12	5/23	15/23	7/8		2/2								
Human convalescent	12/12	4/4			18/18	3/3		1/1	4/4		3/3	3/3		2/2	2/2
Bovine random			34/35	34/35			3/3								
Porcine random	1/1		31/56	54/56			3/3					1/1			
Bovine anti-bovine	3/3	3/3	20/20	20/20	3/3	3/3	3/3	3/3	1/1	2/2	3/3	2/2	1/1	3/3	2/2
Porcine anti-bovine			4/6	6/6			3/3								
Porcine anti-human	5/5		2/12	12/12	1/1		2/2		4/4			2/2			1/1
Porcine anti-equine			1/2	1/2											
Porcine anti-porcine	1/1		0/13	13/13	1/1		3/3		2/2			1/1			1/1
Equine anti-equine	2/2	2/2			2/2	2/2		2/2	2/2	2/2	2/2	2/2		2/2	2/2
Murine anti-murine	1/1	2/2			1/1	1/1		2/2	1/1		2/2	3/3		2/2	2/2
Ovine convalescent		1/1				1/1		1/1			1/1			1/1	

[a] Represents agglutination of rotavirus particles with or without their outer capsid layer (rough or smooth).

TABLE 2. *Antigenic relationship by IEM*

Antiserum	Viral antigen[a]				
	Human	Bovine	Porcine	Murine	Equine
Human	RS	RS	RS	R	RS
Bovine	R	RS	RS	RS	RS
Porcine	R	RS	RS	RS	R
Murine	R	RS	R	RS	R
Porcine anti-human	RS	RS	RS	NT	RS

[a] R, Rough (approximately 56 nm) particles; S, smooth (approximately 65 nm) particles. Antisera agglutinated one or both particles. NT, Not tested.

antibodies for bovine virus, although possessing FA antibody, would develop NT antibodies after several weeks, two pigs were kept in isolators for 8 and 12 weeks, respectively. No alteration was found in their serological response; the sera remained devoid of neutralizing antibodies to the bovine virus.

GD lacked sensitivity as a routine technique for the detection of rotaviruses in fecal extracts prepared for electron microscopy, but was of value in demonstrating antigenic relationships between agents recovered from a number of species. Reactions of identity were observed between rotavirus suspensions derived from infected human cases, an infected mouse, and an infected gnotobiotic calf and with the tissue culture-adapted bovine virus when these were tested in any concentration against human, murine, and gnotobiotic calf rotavirus antisera. Of 148 human fecal samples positive for virus by EM, only 55 were positive by GD.

By GD, antibody to calf rotavirus was detected also in normal human immune globulin, one sample of preserved guinea pig complement, and in a sample of calf serum in routine use for tissue culture purposes. It was not found in acute-phase human sera, preinoculation gnotobiotic calf sera, or in a convalescent sample from a calf infected with reovirus type 3.

By CF tests, sera from convalescent children, calves, and mice reacted with rotavirus antigens prepared from human, bovine, and murine rotaviruses. Serum titers varied between 20 and 80 and were the same for all antigens. However, the optimum peak dilution of each antigen varied with the species of antiserum. Antisera from piglets were not examined since pig serum is known to be a poor fixer of guinea pig complement (3, 4).

Specificity of the bovine virus gnotobiotic antiserum. Standard gnotobiotic antiserum to the bovine virus was shown to have no detectable antibodies to the following bovine and human antigens: (i) by NT or hemagglutin inhibition—bovine coronavirus, reovirus types 1, 2, and 3, bluetongue, bovine viral diarrhea-mucosal disease, parainfluenza 3, rhinovirus SD-1, and respiratory syncytial virus; (ii) by CF tests—influenza A, B, and C, parainfluenza 1 and 3, respiratory syncytial virus, adenovirus group, mumps, herpes simplex, measles, cytomegalovirus, varicella-zoster, *Coxiella burneti*, and chlamydiae.

No cold agglutinins or heterophile antibodies, as demonstrated by the Paul Bunnel test, were present.

Cell culture studies. Immunofluorescent cells were observed in both CK and PK primary cultures and in $IBRS_2$ cell cultures when examined 24 to 48 h after inoculation with fecal filtrates of experimentally passaged bovine virus in calves and pigs or porcine virus passaged in pigs. Equine virus passaged in pigs produced immunofluorescent cells in $IBRS_2$ cells. No difference was observed in the sensitivity of these cells to infection with either bovine or porcine

virus, and the cytoplasmic distribution and appearance of immunofluorescent areas were similar for both viruses. In contrast to the bovine virus, the porcine virus was not adapted to tissue culture, and infectivity was lost after two or three passages in CK and PK cells. Human virus passaged in pigs did not infect PK or CK cells. Immunofluorescent cells were also observed in primary MuK and HEK cell cultures infected with murine virus and in primary HEK and LLC MK_2 cells infected with human, murine, and equine viruses when reacted with antisera to human, bovine, and equine viruses; the pattern of fluorescence observed was similar to that seen in calf kidney cells inoculated with virus from calf feces, except in that the fluorescent granules were smaller. SA.11 and O viruses infected AGMK cells and cross-reacted by immunofluorescence with antisera to human and bovine rotaviruses. Primary cultures and the two cell lines were more susceptible to virus infection than were secondary cell cultures.

DISCUSSION

The morphological similarity and the antigenic relationships of the human, bovine, porcine, murine, and equine viruses provide further evidence for the recognition of these enteric viruses as a distinct group, for which the term rotavirus has been suggested (14). To this group should be added the viruses isolated from the rectal swab taken from a monkey (SA.11) and from the pooled washings from the intestines of sheep and calves (O virus) because their size and shape are similar and because they cross-react by IEM and FA with antisera to human and bovine rotavirus (9). Our findings are in agreement with those of others (19), who used the CF technique to demonstrate an antigenic relationship between human and bovine virus.

The variation observed among randomly selected human and pig antisera in their ability to neutralize the bovine virus, although possessing the fluorescent antibodies for the group antigen, requires further investigation, particularly because this variation was also observed among antisera raised in piglets to the human rotavirus, although not in antisera to the bovine and porcine rotaviruses. One group of naturally infected piglets also showed a variation in the serological response. Only two of seven pigs that showed a serological response by FA also showed an NT response and developed neutralizing antibodies to the calf virus. Great care was taken to avoid laboratory contamination of these viruses, and gnotobiotic piglets were used for all experiments. These precautions appear to have been successful since the human virus infection on passage in piglets remained subclinical and the human and porcine antisera to human virus could be differentiated from antisera to bovine and porcine virus by their ability to agglutinate both "smooth" and "rough" human virus particles.

The lack of a correlation between the agglutination of smooth particles by IEM and neutralizing activity of some antisera may be explained in part by the observation of Bridger and Woode (7). They showed that the infectivity of the virus resided in the smooth particle fraction, but approximately 10^3 particles were required for one infectious unit. If the infectious particle differs from other smooth particles, it is conceivable that agglutination of smooth particles could be accompanied by little loss in infectivity. The group antigen, demonstrable by four serological tests but not by neutralization, may be in both the inner and outer capsid layers of some noninfectious smooth particles, possibly as a result of early viral disintegration, but not in the outer capsid layer of infectious particles.

Thus we are left with at least three explanations for these variations in the activity of antisera: cross-infectivity of viruses between mammalian species, individual variation in the immune response, or the loss of specificity as the immune response matures. The last explanation is unlikely because no alteration in antibody activity was observed in piglets maintained in isolators for 8 and 12 weeks, and the experimental antisera were obtained within 3 weeks of infection. It is probable that cross-infectivity occurs between some mammals because we have shown that piglets can be infected with human, bovine, porcine, and equine "rotaviruses." The neutralization test may be specific for strains of virus because all bovine antisera, obtained either by random selection or from experimentally infected calves, neutralize the bovine virus and possess antibodies reacting in the FA test. If this assumption is correct, it would imply that calves in the United Kingdom are infected predominantly by one strain of virus and children and piglets are infected by two or more distinct strains. However, further differences exist between rotaviruses. The United States isolate of the human rotavirus is pathogenic to pigs and calves (C. A. Mebus, personal communication), whereas the United Kingdon isolate of human rotavirus produced subclinical infection in pigs and failed to infect calves (7a). The United Kingdom isolate of bovine rotavirus is pathogenic for pigs but the United States bovine rotavirus failed to infect pigs (Mebus, personal communication).

Therefore, the variation in the serological response to rotavirus may be a combination of differences between isolates of rotavirus and individual response to the rotavirus.

The presence of a group antigen, demonstrable by FA, IEM, GD, and CF, has proven useful in the diagnosis of virus infection in children, piglets, foals, sheep, goats, rabbits, and guinea pigs, species in which antibodies to the calf virus have been observed. Group-specific antigen and antiserum may be prepared from any one of the rotaviruses and used for diagnosis of infection in any species. No cross-reactivity has been observed with other human and bovine viruses tested.

The morphologically similar viruses found recently in scouring lambs are probably also members of the rotavirus group (23a).

ACKNOWLEDGMENTS

We thank M. J. Dennis for the provision of gnotobiotic animals, Allyson Hawkins and C. Smith for assistance in the electron microscope and serological studies, and P. D. Luther for preparation of cell cultures.

LITERATURE CITED

1. **Banatvala, J. E., B. Totterdell, I. L. Chrystie, and G. N. Woode.** 1975. In-vitro detection of human rotaviruses. Lancet ii:821.
2. **Bishop, R. F., G. P. Davidson, I. H. Holmes, and B. J. Ruck.** 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. Lancet ii:1281-1283.
3. **Blomfield, A. M., R. R. A. Coombs, and N. H. Hole.** 1949. The conglutination phenomenon. V. Further experiments on the importance of the choice of complement when examining antisera for the presence of complement fixing or complement absorbing antibodies. J. Hyg. 47:132-141.
4. **Blomfield, A. M., R. R. A. Coombs, and N. H. Hole.** 1950. The conglutination phenomenon. VI. An experimental investigation of the factors determining the absorption of complement by an antigen-antiserum mixture. J. Hyg. 48:73-95.
5. **Borden, F. C., R. F. Shope, and F. A. Murphy.** 1971. Physicochemical and morphological relationships of some arthropod borne viruses to bluetongue virus—a new taxonomic group; physicochemical and serological studies. J. Gen. Virol. 13:261-271.
6. **Bridger, J. C., and G. N. Woode.** 1975. Neonatal calf diarrhoea: identification of reovirus-like (rotavirus) agent in faeces by immunofluorescence and immune electron microscopy. Br. Vet. J. 131:528-535.
7. **Bridger, J. C., and G. N. Woode.** 1976. Characterization of two particle types of calf rotavirus. J. Gen. Virol. 31:245-250.

7a. **J. C. Bridger, G. N. Woode, J. M. Jones, T. H. Flewett, A. S. Bryden, and H. Davies.** 1975. Transmission of human rotaviruses to gnotobiotic piglets. J. Med. Microbiol. 8:565-569.

8. **Bryden, A. S., and H. A. Davies.** 1975. The laboratory diagnosis of epizootic diarrhoea of infant mice. J. Inst. Anim. Tech. 26:63-67.
9. **Els, H. J., and G. Lecastsas.** 1972. Morphological studies on simian virus SA.11 and the related 'Q' agent. J. Gen. Virol. 17:129-132.
10. **Fernelius, A. L., A. E. Ritchie, L. G. Classick, J. O. Norman, and C. A. Mebus.** 1972. Cell culture adaptation and propagation of a reovirus-like agent of calf diarrhoea from a field outbreak in Nebraska. Arch. Gesamte Virusforsch. 37:114-130.
11. **Flewett, T. H., A. S. Bryden, and H. A. Davies.** 1973. Virus particles in gastroenteritis. Lancet ii:1497.
12. **Flewett, T. H., A. S. Bryden, and H. Davies.** 1974. Diagnostic electron microscopy of faeces. I. The viral flora of the faeces as seen by electron microscopy. J. Clin. Pathol. 27:603-608.
13. **Flewett, T. H., A. S. Bryden, and H. A. Davies.** 1975. Virus diarrhoea in foals and other animals. Vet. Rec. 96:477.
14. **Flewett, T. H., A. S. Bryden, H. Davies, G. N. Woode, J. C. Bridger, and J. M. Derrick.** 1974. Relation between viruses from acute gastroenteritis of children and new born calves. Lancet ii:61-63.
15. **Flewett, T. H., H. A. Davies, A. S. Bryden, and M. J. Robertson.** 1974. Diagnostic electron microscopy of faeces. II. Acute gastroenteritis associated with reovirus-like particles. J. Clin. Pathol. 27:608-614.
16. **Grist, N. R., C. A. C. Ross, E. J. Bell, and E. J. Stott.** 1966. Diagnostic methods in clinical virology, p. 54-71. Blackwell Scientific Publications, Oxford.

16a. **Hall, G. A., J. C. Bridger, R. L. Chandler, and G. N. Woode.** 1976. Gnotobiotic piglets experimentally infected with neonatal calf diarrhea reovirus-like agent (Rotavirus). Vet. Pathol. 13:197-210.

17. **Holmes, I. H., B. J. Ruck, R. F. Bishop, and G. P. Davidson.** 1975. Infantile enteritis viruses: morphogenesis and morphology. J. Virol. 16:937-943.
18. **Kapikian, A. Z., W. L. Cline, C. A. Mebus, R. G. Wyatt, A. R. Kalica, H. D. James, D. Van-Kirk, R. M. Chanock, and H. W. Kim.** 1975. New complement fixation test for the human reovirus-like agent of infantile gastroenteritis. Lancet i:1056-1061.
19. **Kapikian, A. Z., H. W. Kim, R. G. Wyatt, W. J. Rodriguez, S. Ross, W. L. Cline, R. H. Parrott, and R. M. Chanock.** 1974. Reovirus-like agent in stools: association with infantile diarrhoea and development of serologic tests. Science 185:1049-1053.
20. **Lecatsas, G. J.** 1974. Virus particles in acute gastroenteritis. Lancet ii:524.
21. **Middleton, P. J., M. T. Szymanski, G. D. Abbott, R. Bartolussi, and J. R. Hamilton.** 1974. Orbivirus acute gastroenteritis of infancy. Lancet i:1241-1244.
22. **Much, D. H., and I. Zajac.** 1972. Purification and characterization of epizootic diarrhea of infant mice virus. Infect. Immun. 6:1019-1024.

22a. **Newman, F. E., F. Brown, J. C. Bridger, and G. N. Woode.** 1975. Characterization of rotaviruses. Nature (London) 258:631-633.

23. **Rodger, S. M., R. D. Schnagl, and I. H. Holmes.** 1975. Biochemical and biophysical characteristics of diarrhea viruses of human and calf origin. J. Virol. 16:1229-1235.

23a. **Snodgrass, D. R., W. Smith, E. W. Gray, and J. A. Herring.** 1976. A rotavirus in lambs with diarrhea. Res. Vet. Sci. 20:113-114.

24. **Stair, E. L., M. B. Rhodes, R. G. White, and C. A. Mebus.** 1972. Neonatal calf diarrhoea: purification and electron microscopy of a coronavirus-like agent. Am. J. Vet. Res. 33:1147-1156.
25. **Tavernor, W. D., P. C. Trexler, L. C. Vaughan, J. E. Cox, and D. G. C. Jones.** 1971. The production of gnotobiotic piglets and calves by hysterotomy under general anaesthesia. Vet. Rec. 88:10-14.
26. **Woode, G. N., and J. C. Bridger.** 1975. Viral enteritis of calves. Vet. Rec. 96:85-88.
27. **Woode, G. N., J. C. Bridger, G. A. Hall, and M. J. Dennis.** 1974. The isolation of a reovirus-like agent associated with diarrhoea in colostrum derprived calves in Great Britain. Res. Vet. Sci. 16:102-104.

27a. **Woode, G. N., J. C. Bridger, G. A. Hall, J. M. Jones, and G. Jackson.** 1976. The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. J. Med. Microbiol. 9:203-209.