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Detection of Group C Rotavirus Antigens and Antibodies in Animals and Humans by Enzyme-Linked Immunosorbent Assays†

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Enzyme-linked immunosorbent assays (ELISAs) were developed to detect group (gp) C rotavirus antigens and antibodies. Both assays were confirmed to be specific for gp C rotavirus by using serogroup A, B, and C rotaviruses; hyperimmune antisera to these serogroups of rotaviruses; and paired serum specimens from animals infected with gp C rotaviruses. The ELISA for antigen detection reacted not only with porcine gp C rotaviruses but also with human and bovine gp C rotaviruses. Following experimental challenge of gnotobiotic pigs with porcine gp C rotavirus, the virus was found by ELISA in all diarrheic feces. A high prevalence of antibodies to gp C rotaviruses was detected in sera from adult pigs (93 to 97%) and cattle (47 to 56%) in the United States and Japan. However, no antibody to gp C rotavirus was detected in the sera (n = 20) of adult horses in the United States. In human sera from Hokkaido, Japan, 3% of children and 13% of adults possessed antibody to gp C rotaviruses. These results suggest that the ELISA that we developed may be useful for surveying gp C rotavirus infections in animals and humans. On the basis of serology, gp C rotavirus infections are common in pigs and cattle in the United States and Japan, but they occur at lower levels in humans from the Hokkaido area of Japan.

Rotaviruses are a major cause of diarrhea in animal and human neonates (13, 19). They are classified as a genus within the family *Reoviridae* on the basis of a characteristic morphology that comprises double-shelled particles surrounding a genome of 11 segments of double-stranded RNA (dsRNA). Rotaviruses are assigned to seven serogroups (A to G) on the basis of serology and genome analysis (20, 25, 26, 28, 33, 36). The dsRNA segments of each group (gp) of rotavirus show similar electrophoretic mobilities, and each rotavirus serogroup contains common or cross-reactive antigens.

The gp C rotaviruses have been detected in swine (1, 9, 10, 14, 21, 30), humans (6, 8, 11, 18, 27, 45), and recently, cattle (43). Porcine gp C rotaviruses cause diarrhea in experimentally inoculated gnotobiotic pigs (1, 15, 38), and limited serological surveys have shown that gp C rotaviruses are prevalent in pigs in North America, Australia, and Europe (4, 21, 28, 38). Recently, large-scale outbreaks of diarrhea in children caused by gp C rotaviruses have been documented in Japan (18). However, the etiologic role and the global distribution of gp C rotaviruses in human and animal diarrhea outbreaks remain unclear.

Immune electron microscopy and polyacrylamide gel electrophoresis of viral dsRNA have been the primary methods of identifying gp C rotavirus infections (6, 14, 18, 21, 27, 28, 30, 33, 43, 45). However, no large-scale epidemiologic studies have been done because the inability to cultivate most gp C rotaviruses has hampered the preparation of diagnostic reagents. Recently, porcine (Cowden) and bovine (Shintoku) strains of gp C rotavirus have been propagated in a monkey kidney cell line (MA104) (32, 43). The objectives of this study were to develop sandwich and blocking enzyme-linked immunosorbent assays (ELISAs) to detect gp C rotavirus antigens and antibodies, respectively, by using a hyperimmune antiserum to the tissue culture-adapted gp C rotavirus (Cowden). We then used the blocking ELISA to investigate the seroepidemiology of gp C rotavirus infections in humans and animals.

MATERIALS AND METHODS

Rotaviruses. The Cowden strain of porcine gp C rotavirus was propagated in MA104 cells and gnotobiotic pigs (1, 15, 32). Five non-cell-culture-adapted field strains of porcine gp C rotaviruses, each having a distinct dsRNA electropherotype and designated WH, KH, Ah, NB and Wi, were propagated in gnotobiotic pigs (15, 38). The Shintoku strain of bovine gp C rotavirus was propagated in a colostrum-deprived calf. Human gp C rotaviruses (88-182, 88-196, and 88-261 strains) were supplied by M. Oseto and Y. Yamashita of the Ehime Prefectural Institute of Public Health, Ehime, Japan. The porcine (OSU and Gottfried strains), human (Wa strain), and bovine (NCDV strain) gp A rotaviruses and porcine (Ohio strain) and bovine (ATI strain) gp B rotaviruses were propagated in gnotobiotic pigs or calves (17, 28, 33, 40). The propagation of these viruses in animals or humans was confirmed by immune electron microscopy (29). Intestinal or fecal contents were diluted to produce 2.5% (wt/vol) suspensions with 10 mM phosphate-buffered saline (PBS; pH 7.4) containing 0.5% bovine serum albumin (BSA), 2% fetal calf serum, and 0.1% Tween 20. The suspensions were clarified by centrifugation at 650 × g for 30 min at 4°C, and the supernatants were used for ELISA. Preexposure fecal samples collected from 10 gnotobiotic pigs were treated as described above and were used as negative controls. The Cowden gp C rotavirus was propagated in MA104 cells and purified on CsCl gradients, and its protein concentration was

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determined (16). The virus was serially diluted in the diluent described above, and the solution was titrated to determine the sensitivity of the ELISA for detecting the homologous gp C rotavirus antigen.

Hyperimmune antisera. Hyperimmune antisera to bovine gp A (NCDV), porcine gp A (OSU, Gottfried), porcine gp B (Ohio), bovine gp B (ATI), porcine gp C (Cowden), and bovine gp C (Shintoku) rotaviruses and transmissible gastroenteritis virus were prepared in gnotobiotic pigs, gnotobiotic calves, or guinea pigs as described previously (1, 2, 28, 32, 33, 38, 40). Preexposure sera from five gnotobiotic pigs and five colostrum-deprived calves were used as antibody-negative controls. Immunoglobulin G was purified from an antiserum (designated U339) to tissue culture-adapted Cowden gp C rotavirus by using a protein A-Sepharose column and was labeled with biotin for ELISA (12, 35).

Experimental inoculations. Four gnotobiotic pigs (3 to 6 days old) were orally inoculated with Cowden gp C rotavirus passaged several times in gnotobiotic pigs (1). A colostrum-deprived calf (1 day old) was orally inoculated with gp C rotavirus (fecal filtrate) derived from a naturally infected cow (43). Rectal swabs from the pigs and feces from the calf were collected daily and clinical signs were noted. In addition, fecal samples were collected daily from three colostrum-deprived calves after inoculation with NCDV bovine gp A rotavirus, and the fecal samples were confirmed to be positive for rotavirus shedding for 4 to 6 days after inoculation (41, 44). Rectal swab fluids from the pigs were considered as a 1:25 dilution and were processed as described previously (31, 38). Fecal suspensions (2.5%) from the calves were prepared as described above. These samples were examined for gp C rotavirus by ELISA and a cell culture immunofluorescent (CCIF) test (38).

Serum samples. A total of 405 serum samples obtained from four different species were tested for antibodies to gp C rotavirus. From cattle, 92 serum samples were analyzed, including (i) 4 paired serum samples from cattle with field gp C rotavirus infections in Hokkaido, Japan, (ii) 1 paired serum sample from a colostrum-deprived calf that was experimentally inoculated with Shintoku gp C rotavirus, and (iii) 82 serum samples from adult beef and diary cows (32 serum samples from herds in Ohio, Tennessee, and Virginia and 50 serum samples from 20 herds in Hokkaido, Japan). The 156 porcine serum samples included (i) 4 paired serum samples from pigs experimentally inoculated twice with porcine gp C rotaviruses (the first challenge was with the Cowden, WH, or Wi strain and the second challenge was with the Cowden or Wi strain) and (ii) 148 serum samples from adult pigs (68 serum samples from five swine herds in Ohio and South Dakota and 80 serum samples from seven swine herds in Hokkaido, Japan). The 20 equine serum samples were from adult thoroughbred horses from four farms in Kentucky. The 137 human serum samples included (i) 6 convalescent-phase serum samples from patients with human gp C rotavirus gastroenteritis in Japan (kindly supplied by H. Ushijima, National Institute of Health, Tokyo, Japan) and (ii) 78 randomly selected serum samples from healthy children (mean age, 6 years; age range, 2 months to 15 years) and 53 randomly selected serum samples from healthy adults (mean age, 56 years; age range, 20 to 84 years) in Hokkaido, Japan, where human gp C rotavirus outbreaks have not been identified (kindly supplied by K. Furuya and H. Sawada, Hokkaido Prefectural Institute of Public Health, Sapporo, Japan).

ELISA for the detection of gp C rotavirus antigens. The ELISA used for detecting gp C rotavirus antigens was a sandwich assay with a hyperimmune gnotobiotic pig serum (U339) to Cowden gp C rotavirus as both the capture and the biotinylated detector antibody.

Microplates (MaxiSorp; Nunc, Inc., Naperville, Ill.) were coated overnight at 4°C with 100 μl (per well) of a 1:3,000 dilution of either the hyperimmune antiserum (U339) or a preimmune gnotobiotic pig serum diluted in carbonate-bicarbonate buffer (pH 9.6). The plates were washed with PBS containing 0.1% Tween 20 (PBS-T) and blocked with 2% BSA in PBS at room temperature for 1 h. After washing with PBS-T, 100 μl of test samples was added and the plates were incubated at 37°C for 2 h. Biotinylated U339 immunoglobulin G at a concentration of 1 μg/ml in 0.1 M bicarbonate buffer (pH 8.2) containing 0.01 M NaCl and 0.002 M KCl was added to each well of the washed plates, which were then incubated at 37°C for 1 h. After washing with PBS-T, 100 μl (per well) of horseradish peroxidase-conjugated streptavidin (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) diluted 1:4,000 in PBS-T was added. The plates were incubated at room temperature for 30 min and washed with PBS-T. Two hundred microliters of 2,2′-azino-bis(3-ethylbenthizoline-6-sulfonate) and 0.045% H_2O_2 in 0.1 M sodium citrate buffer (pH 4.2) was added to each well, and the plates were developed at room temperature for 30 min. The reaction was stopped by adding 5% sodium dodecyl sulfate. The A_{414} was measured with a micro-ELISA reader and was expressed as the ratio of the A_{414} in the wells coated with hyperimmune antiserum (U339) to the A_{414} in the wells coated with preexposure serum (P/N ratio).

Blocking ELISA. Antibodies to gp C rotaviruses were measured by a blocking ELISA. One hundred microliters of Cowden gp C rotavirus propagated in MA104 cells (producing an A_{414} of approximately 1.0 in the antigen ELISA system) was added to wells that were coated with the U339 antiserum and blocked with 2% BSA in PBS. The plates were incubated at 37°C for 2 h, and after washing with PBS-T, serial twofold dilutions of test sera (initial dilution, 1:10) in PBS-T containing 2% fetal calf serum and 0.5% BSA were added. After 1 h of incubation at 37°C, the assay was continued as described above for the antigen ELISA, with the addition of biotinylated gp C rotavirus antiserum (U339) as the detector antibody.

A blocking ELISA for detecting antibodies to gp A rotavirus was developed by modifying the ELISA for gp A rotavirus antigen described previously (41). Briefly, microplates were coated with a hyperimmune guinea pig antiserum to NCDV gp A rotavirus and blocked with 1% BSA. Sera were serially diluted twofold, mixed with an equal volume of purified NCDV gp A rotavirus, and incubated at 4°C for 16 h; and the mixture was then added to the plates and incubated at 37°C for 2 h. Viral antigen was detected with a monoclonal antibody that was reactive with a common gp A rotavirus antigen; this was followed by the addition of anti-mouse immunoglobulin conjugated with peroxidase. The color reaction was developed as described above.

Antibody titers to gp C and A rotaviruses were expressed as the reciprocal of the highest dilution of each serum sample which showed at least a 50% reduction of the absorbance when it was compared with the absorbance of the buffer control.

CCIF tests for detection of gp C rotavirus antigens and antibodies. Rectal swabs collected from gnotobiotic pigs experimentally inoculated with porcine gp C rotavirus (Cowden) were processed, and the fluids were examined for gp C rotavirus by a CCIF test by using MA104 cells, as described

TABLE 1. Specificity of the ELISA for distinguishing gp C rotaviruses from gp A and B rotaviruses

Host species[a]	Serogroup	Strain	A_{414}	P/N ratio[b]
Porcine	C	Cowden	0.484	12.7
Porcine	C	WH	1.996	24.3
Porcine	C	KH	0.126	4.6
Porcine	C	Ah	0.355	10.4
Porcine	C	NB	2.732	6.1
Porcine	C	Wi	0.139	4.6
Bovine	C	Shintoku	0.766	8.3
Human	C	88-182	0.431	7.0
Human	C	88-196	2.985	46.5
Human	C	88-261	0.467	8.1
Porcine	A	OSU	0.041	1.5
Porcine	A	Gottfried	0.068	0.9
Bovine	A	NCDV	0.070	1.1
Human	A	Wa	0.066	1.2
Porcine	B	Ohio	0.055	0.9
Bovine	B	ATI	0.075	0.9
Negative feces[c]			0.044	1.1

[a] Fecal samples were from experimentally inoculated gnotobiotic pigs or a colostrum-deprived calf (Shintoku gp C) or from naturally infected humans (88-182, 88-196, and 88-261 gp C).

[b] The negative (N) value (not shown) for calculation of the P/N ratio was the A_{414} obtained for each sample with preexposure gnotobiotic pig serum as the capture antibody.

[c] Feces were collected from 10 gnotobiotic pigs before they were exposed to gp C rotavirus. The A_{414} and the P/N ratio shown (negative feces) are the mean values from these samples. Samples with an A_{414} of ≥0.09 and a P/N ratio of ≥1.7 were considered positive for gp C rotavirus.

previously (38). For measuring antibody titers to gp C and A rotaviruses in serum, MA104 cells infected with Cowden gp C or NCDV gp A rotavirus were used in an indirect immunofluorescence (IF) test (37, 38, 43).

RESULTS

Specificity and sensitivity of the ELISA for detecting gp C rotavirus. The virus-negative fecal samples collected from 10 preexposure gnotobiotic pigs showed a mean A_{414} of 0.044 with a standard deviation of 0.015 and a mean P/N ratio of 1.07 with a standard deviation of 0.19 (Table 1). Samples were considered positive for gp C rotavirus if the A_{414} was ≥0.09 (mean + 3 standard deviations) and the P/N ratio was ≥1.7 (mean + 3 standard deviations). All 10 fecal samples containing gp C rotaviruses confirmed by immune electron microscopy tested positive for gp C rotavirus in the ELISA, regardless of the strain or species of origin. However, none of the fecal samples containing gp A or B rotaviruses tested positive in the ELISA (Table 1).

All four pigs inoculated with Cowden gp C rotavirus became diarrheic on postinoculation days 2 to 5, and the diarrhea persisted for 2 to 6 days. The gp C rotavirus was detected in the feces by ELISA on postinoculation days 1 to 4, and shedding persisted for 3 to 7 days. All the diarrheic fecal samples were positive for gp C rotavirus by ELISA; however, several of these fecal samples were negative for gp C rotavirus by the CCIF test (Table 2). One calf challenged with Shintoku gp C rotavirus became diarrheic on postinoculation day 2 and remained diarrheic for 2 days. Virus shedding was detected by ELISA on day 2 and persisted for 4 days. All fecal samples from calves challenged with gp A rotavirus were negative for gp C rotavirus by ELISA (data not shown).

The sensitivity of the ELISA was evaluated by testing serial dilutions of purified Cowden gp C rotavirus. The ELISA detected 40 ng of the purified virus per ml.

TABLE 2. Virus detection by ELISA and the CCIF test in feces of gnotobiotic pigs and a colostrum-deprived calf inoculated with the Cowden and Shintoku gp C rotaviruses, respectively

Pig or calf no. (age [days])	Diarrhea		Virus detection by:			
			CCIF test		ELISA	
	PID[a] of onset	Duration (days)	PID of onset	Duration (days)	PID of onset	Duration (days)
Pig 1 (4)	5	2	4	1	4	3
Pig 2 (4)	2	6	4	1	1	7
Pig 3 (3)	2	3	2	3	2	5
Pig 4 (6)	4	2	2	6	2	5
Calf 1 (1)	2	2	NT[b]	NT	2	4

[a] PID, postinoculation day.

[b] NT, not tested.

Specificity and sensitivity of the blocking ELISA for detecting gp C rotavirus antibody. The specificity of the blocking ELISA for antibody detection was evaluated with 12 hyperimmune antiserum specimens prepared against three serogroups of rotaviruses (Table 3). Minor cross-reactivities between gp A and gp C rotaviruses were found by the indirect IF test, as we have described previously (43). On the other hand, none of the hyperimmune antisera to gp A and C rotaviruses reacted with the heterologous serogroup of rotavirus in the blocking ELISA (Table 3). Hyperimmune antiserum to transmissible gastroenteritis virus and preexposure sera collected from five gnotobiotic pigs and five colostrum-deprived calves were also negative for antibodies to both gp A and C rotaviruses in the ELISA.

The specificity and sensitivity of the blocking ELISA were also examined by using sera collected from animals that were experimentally or naturally infected with gp C rotavirus (Fig. 1). All acute-phase sera tested negative for gp C rotavirus antibodies; however, significant antibody levels (fourfold or greater) to gp C rotavirus were detected in all convalescent-phase sera by the blocking ELISA. After the second experimental challenge of the pigs with rotavirus, a 128-fold or greater increase in antibodies to gp C rotavirus in serum was detected. All five of the convalescent-phase sera collected from patients who were naturally infected with human gp C rotavirus tested positive for gp C rotavirus antibody, with titers ranging from 10 to 40 (Fig. 1). All convalescent-phase sera from gp C rotavirus-inoculated gnotobiotic pigs and a calf were negative for gp A rotavirus antibodies (antibody titers, <10).

Prevalence of antibodies to gp C rotavirus. A total of 381 serum samples from four different species were tested for gp C rotavirus antibodies (Table 4). A high prevalence of antibody to gp C was found in adult pig sera (97% in the United States and 93% in Japan). The geometric mean antibody titers (GMT; 59 to 120) in adult pig sera were significantly higher than those in the other species tested. Some 47% of bovine sera from the United States and 56% of bovine sera from Japan tested positive for gp C rotavirus antibodies; the antibody titers were relatively low (GMT, 17 to 27). Thirteen percent of sera from human adults tested positive for antibodies to gp C rotavirus. However, only two serum samples (3%) from children tested positive. None of the equine sera had antibodies to gp C rotavirus (GMT, <10). Five serum samples that were highly positive for gp C rotavirus antibodies and five serum samples that were neg-

TABLE 3. Antibody titers to gp A and C rotaviruses in serogroup-specific hyperimmune antisera by the blocking ELISA and indirect IF test

Hyperimmune antisera				Antibody titers[a] to rotavirus by:			
				Blocking ELISA		Indirect IF	
Immunized animal[b]	Host species, serogroup	Rotavirus strain	Designation	gp A	gp C	gp A	gp C
GN[b] pig	Bovine, A	NCDV	T1516	10,240	<10	81,920	160
GN pig	Bovine, A	NCDV	I655	NT[c]	<10	16,000	160
Guinea pig	Bovine, A	NCDV	AN1	5,120	<10	5,120	160
GN pig	Porcine, A	OSU	F509	NT	<10	81,920	320
Guinea pig	Porcine, A	OSU	8807D	2,560	<10	NT	NT
GN pig	Porcine, A	Gottfried	N926	320	<10	20,480	80
GN pig	Porcine, B	Ohio	P66	<10	<10	<10	<10
GN calf	Bovine, B	ATI	H14	<10	<10	<10	<10
GN pig	Porcine, C	Cowden	U340	<10	2,560	<160	5,120
GN pig	Porcine, C	Cowden	J33	<10	10,240	80	10,240
GN pig	Porcine, C	Cowden	W540	<10	40,960	80	20,480
Guinea pig	Bovine, C	Shintoku	CS1	<10	320	160	10,240

[a] Antibody titers represent the reciprocal of the highest antiserum dilution that showed a positive reaction to group A (NCDV) or group C (Cowden) rotaviruses in the respective assays.
[b] GN, gnotobiotic.
[c] NT, not tested.

ative by the blocking ELISA were selected from each of three species (pigs, cattle, and humans) and tested for antibodies to gp A rotavirus by ELISA. The GMT of antibodies to gp C rotavirus in the positive sera were 724 in pigs, 69 in cattle, and 69 in humans. All sera were positive for gp A rotavirus antibodies, and there was no significant difference in the GMT of antibodies to gp A rotavirus between sera that were positive and those that were negative for gp C rotavirus antibodies in each species (data not shown).

DISCUSSION

Recently, researchers have developed an ELISA for detecting non-gp A rotavirus antigens and antibodies, in particular, the gp B rotaviruses, by using polyclonal or monoclonal antibodies (7, 22, 46). Concerning gp C rotaviruses, Debouck et al. (10) used an ELISA to monitor the presence of porcine gp C rotavirus in closed swine herds, but the assay was not applied to the detection of non-porcine gp C rotaviruses. A monoclonal antibody-based ELISA has been developed for antigen detection, but only a few non-porcine gp C rotavirus strains were tested in the assay (23, 24). To our knowledge, there are no reports that have described an ELISA that can be used for the detection of gp C rotavirus antibodies. The antigen ELISA developed in the study described here was highly specific for gp C rotaviruses, with no reactions to the various strains of gp A and B rotaviruses tested. The assay detected all strains of gp C rotaviruses in feces collected from pigs, cattle, and humans. The ELISA detected as little as 40 ng of the homologous viral protein per ml. This corresponds to approximately 10^8 particles per ml and is equivalent in sensitivity to that of electron microscopy (34). After experimental inoculation of pigs and a calf with gp C rotaviruses, viral shedding was confirmed in all diarrheic feces by ELISA but not by the CCIF test. These results suggest that this ELISA may be suitable for the investigation of gp C rotavirus infections in humans and animals, allowing the screening of large numbers of fecal samples.

The blocking ELISA for the detection of antibody was also specific to gp C rotaviruses. All convalescent-phase sera from humans, cattle, and pigs experimentally or naturally infected with gp C rotaviruses were positive for antibodies to gp C rotaviruses, although most titers were low. Possible explanations for the low antibody titers are as follows. First, infections with gp C rotaviruses may be more transient than infections with gp A rotaviruses, and thus, they may fail to induce high levels of serum antibody. Second, the pattern of infections may be different from those of gp A rotaviruses, as suggested by Theil and Saif (39) for gp B rotavirus infections. Finally, the sensitivity of the blocking ELISA was no higher than that of a similar assay for antibodies to gp A rotavirus reported previously (47). The antibody titer of the antiserum to Shintoku gp C rotavirus (designated CS1) determined by the blocking ELISA was lower than that of antisera to Cowden gp C rotavirus (designated J33), although their antibody titers in the indirect IF test were the same when cells infected with either Cowden or Shintoku gp C rotaviruses were used (43). These results suggest that significant antigen variations may occur in the blocking ELISA; these variations may arise from possible different subgroups or serotypes within gp C rotaviruses. Since Cowden gp C rotavirus was used to coat the plates, the blocking ELISA probably detected antibodies both to the major group antigens (Cowden and Shintoku strains) and to other unique antigens (Cowden strain specific) within gp C rotaviruses, accounting for the antibody titer differences that we observed.

Minor cross-reactivities between gp A and gp C rotaviruses have been observed in the indirect IF test (43). Recently, we have produced and characterized monoclonal antibodies that are reactive to both serogroups of rotavirus, confirming that the cross-reactions in the indirect IF test were specific (42). These monoclonal antibodies recognized both VP6 of gp A rotaviruses and the 41-kDa protein (VP6 counterpart) of gp C rotaviruses (3, 16). These results suggest that minor common epitopes exist on the VP6 protein of gp A and C rotaviruses. ELISA failed to show cross-reactivity with gp A rotavirus antigens or antibodies. It may be that antibodies to these common epitopes between rotavirus serogroups A and C are rare in most convalescent-phase and hyperimmune sera. Therefore, dilution of the capture and detector antibodies used for the ELISA probably eliminated the minor cross-reacting antibodies, such that

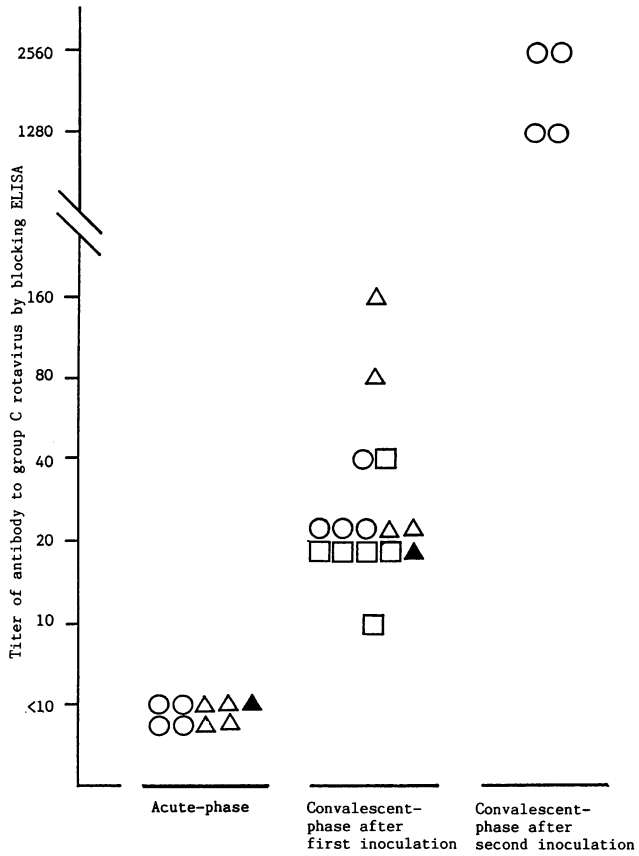

FIG. 1. Blocking ELISA antibody titers to gp C rotaviruses of sera from humans, pigs, and cattle experimentally or naturally infected with gp C rotaviruses. Symbols: □, sera from humans naturally infected with gp C rotaviruses; ○, sera from pigs experimentally infected with gp C rotaviruses; △, sera from cows naturally infected with gp C rotavirus; ▲, serum from a calf experimentally infected with gp C rotavirus.

only gp C rotavirus-specific antigen and antibody reactions were observed.

In the study described here, a very high prevalence of antibodies to gp C rotavirus was found in sera from adult pigs in both Japan and the United States. This finding agrees with the results of other investigations in the United Kingdom, Australia, and the United States (4, 21, 28, 33, 38). The gp C rotaviruses may play an important role in the etiology of porcine diarrhea in the field, since various strains of porcine gp C rotaviruses induce diarrhea in experimentally inoculated gnotobiotic pigs, as confirmed in this study (1, 15, 38). A high prevalence of gp C rotavirus antibodies in sera from cattle from Japan and the United States was also recognized in the study described here. Although antibodies to gp C rotavirus were reported in the sera of cattle in the United Kingdom (5) and the United States (28), gp C rotaviruses have been isolated only from cattle in Japan (43). Further studies are needed to clarify the epidemiology of gp C rotavirus infections and to define their role in diarrheal diseases of cattle.

There have been recent reports of outbreaks of diarrhea associated with gp C rotaviruses in humans in Japan (18, 45). In the study described here, we used human sera collected in Hokkaido Prefecture, Japan, where human gp C rotaviruses have not been identified. A low prevalence of antibodies to gp C rotavirus was detected in the sera of individuals who live in that region of Japan (3% of children and 13% of adults). This result is similar to those presented in previous reports of limited serological surveys in Mexico and the United Kingdom (6, 11, 28). Therefore, it is possible that gp C rotavirus infections occur at a low level in humans worldwide. There are no reports on the interspecies transmission of gp C rotaviruses; however, zoonotic infections of humans with gp C rotaviruses from pigs or cattle cannot be excluded at this time.

TABLE 4. Prevalence and titers of antibody to gp C rotaviruses in sera from pigs, cattle, horses, and humans in the United States and Japan

Species	Location	No. tested	No. (%) positive	GMT[a]
Pig	United States	68	66 (97.1)	59
Pig	Japan	80	74 (92.5)	120
Cattle	United States	32	15 (46.9)	17
Cattle	Japan	50	28 (56.0)	27
Horse	United States	20	0 (0)	<10
Human (child[b])	Japan	78	2 (2.6)	20
Human (adult[c])	Japan	53	7 (13.1)	44

[a] GMT to gp C rotavirus of the antibody-positive sera.
[b] The mean age was 6 years, and the age range was from 2 months to 15 years.
[c] The mean age was 56 years and age range was from 20 to 84 years.

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