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Neutralizing Antibody to Calf Diarrhea Coronavirus in Various Animal Species in Japan

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Coronavirus infections associated with respiratory, intestinal or other diseases have been reported in a number of animal species, including man, cattle, pig, dog, cat, mouse, rat, turkey, and chicken (1–3, 5, 6, 8, 10, 12, 13). Recently coronaviruses have been divided by immunofluorescence into two distinct groups on the basis of antigenic cross-reactivity (9), although coronaviruses, as a group, display complex serologic variability (5). In this paper we report on the presence of neutralizing antibodies against calf diarrhea coronavirus (6, 12) in serum specimens from various animal species.

Calf diarrhea coronavirus passaged in bovine embryonic kidney cells (6) was kindly supplied by Dr. C.A. Mebus, University of Nebraska, U.S.A. In our laboratory the virus was shown to replicate readily and induce a cytopathic effect in cultures of a continuous cell line, BEK-1, derived from bovine embryonic kidney (4). In the present study the virus was used at the 7th passage level in BEK-1 cells.

The neutralization test was carried out by the serum dilution method in 11 × 100-mm tube cultures of BEK-1 cells. The growth medium was Eagle's minimum essential medium (MEM) containing 10% tryptose phosphate broth (TPB) and 10% inactivated calf serum, and the maintenance medium was MEM containing 10% TPB, 0.05% yeast extract, 0.5% sodium glutamate and 0.1% glucose. Serial fourfold dilutions of the serum inactivated at 56 C for 30 min were made in maintenance medium and mixed with equal volumes of maintenance medium containing 200 $TCID_{50}$ of virus per 0.1 ml. The serum-virus mixtures were incubated at 37 C for 1 hr before inoculation of 0.1-ml volumes into two tube cultures per serum dilution. The tests were read after incubation in a roller drum at 37 C for 4 days. The antibody titer was expressed as the reciprocal of the highest serum dilution which showed complete neutralization in at least one of the two tubes. Titers of 2 or higher were taken as positive.

A total of 306 serum samples were collected from 12 animal species in various parts of Japan during the period from 1975 to 1977 (Table 1). These samples were stored at −20 C and inactivated at 56 C for 30 min before being tested.

The results of neutralization tests on these serum samples are summarized in

Table 1. Distribution of titers of neutralizing antibody to bovine coronavirus in various animal species

Species	Age	No. tested	Positive %	No. of animals with NT titer of						
				<2	2	8	32	128	512	≥2,048
Cattle	Adult	20	100	0	0	0	2	4	11	3
Horse	5–6 yr	20	90	2	5	8	3	2		
Sheep	Adult	20	85	3	6	10	1			
Goat	Adult	20	0	20						
Pig	Adult	59	75	15	2	1	7	9	7	18
Rabbit	Adult	20	35	13	4	3				
Guinea pig	Adult	27	22	21	0	3	2	1		
Rat	Adult	20	100	0	0	2	2	8	4	4
Hamster	Adult	20	0	20						
Mouse	6 weeks	40	45	22	13	5				
Chicken	3–5 yr	20	0	20						
Human	Adult	20	100	0	0	4	11	5	1	

Table 1. Neutralizing antibody to calf diarrhea coronavirus was detected in serum samples from all species except goat, hamster, and chicken. The incidence of antibody in titers of 2 or higher ranged from 22 to 100%, and high incidences, exceeding 70%, were obtained in cattle, horses, sheep, pigs, rats, and human beings. High titers were detected especially in cattle, pigs, and rats, some animals having titers of even 2,048 or higher. Similar high incidences of neutralizing and hemagglutination-inhibiting antibodies to calf diarrhea coronavirus have been reported in normal adult cattle and pigs (11).

These serological results suggest that calf diarrhea coronavirus or antigenically related virus(es) occur commonly in these animal species in Japan. The present observation that swine and rat sera, like bovine sera, showed not only high antibody incidence but also frequent high antibody titers for calf diarrhea coronavirus may suggest close antigenic relationships between calf diarrhea coronavirus and coronaviruses occurring in pigs and rats. However, our recent study (11) showed that there is a marked antigenic difference between calf diarrhea coronavirus and hemagglutinating encephalomyelitis virus of swine (7), although there is some cross reaction. There is an urgent need to conduct a systematic comparative study on the antigenic composition of coronaviruses.

These results seem to provide serological evidence for the occurrence of coronavirus infection in horses, sheep, rabbits, and guinea pigs, in which no report on the occurrence of coronavirus is available.

REFERENCES

1) Bass, E.P., and Sharpee, R.L. 1975. Coronavirus and gastroenteritis in foals. Lancet **2**: 822.
2) Binn, L.N., Lazar, E.C., Keenen, K.P., Huxsoll, D.L., Marchwicki, R.H., and Strano, A.J. 1974. Recovery and characterization of a coronavirus from military dogs with diarrhea. Proc. U.S. Animal Health Assoc. **78**: 356–366.
3) Horzinek, M.C., Osterhaus, A.D.M.E., and Ellens, D.J. 1977. Feline infectious peritonitis virus. Zbl. Vet. Med. B **24**: 398–405.

4) Inaba, Y., Sato, K., Kurogi, H., Takahashi, E., Ito, Y., Omori, T., Goto, Y., and Matumoto, M. 1976. Replication of bovine coronavirus in cell line BEK-1 culture. Arch. Virol. **50**: 339–342.
5) McIntosh, K. 1974. Coronaviruses: a comparative review. Curr. Top. Microbiol. Immunol. **63**: 85–129.
6) Mebus, C.A., Stair, E.L., Rhodes, M.B., and Twiehaus, M.J. 1973. Neonatal calf diarrhea: propagation, attenuation and characteristics of a coronavirus-like agent. Am. J. Vet. Res. **34**: 145–150.
7) Mengeling, W.L., Boothe, A.D., and Ritchine, A.E. 1972. Characteristics of a coronavirus (strain 67N) of pigs. Am. J. Vet. Res. **33**: 297–308.
8) Pedersen, N.C. 1976. Morphologic and physical characteristics of feline infectious peritonitis virus and its growth in autochthonous peritoneal cell cultures. Am. J. Vet. Res. **37**: 567–572.
9) Pedersen, N.C., Ward, J., and Mengeling, W.L. 1978. Antigenic relationship of the feline infectious peritonitis virus to coronaviruses of other species. Arch. Virol. **58**: 45–53.
10) Ritchine, A.E., Desmukh, D.R., Larsen, C.T., and Pomeroy, B.S. 1973. Electron microscopy of coronavirus-like particles characteristic of turkey bluecomb disease. Avian Dis. **17**: 546–558.
11) Sato, K., Inaba, Y., and Matumoto, M. 1980. Serological relation between calf diarrhea coronavirus and hemagglutinating encephalomyelitis virus. Arch. Virol. **66**: 157–159.
12) Sharpee, R.L., Mebus, C.A., and Bass, E.P. 1976. Characterization of a calf diarrheal coronavirus. Am. J. Vet. Res. **37**: 1031–1041.
13) Tyrrell, D.A.J., Almeida, J.D., Cunningham, C.H., Dowdle, W.R., Hofstad, M.S., McIntosh, K., Tajima, M., Zakstalskaya, L.Y., Easterday, B.C., Kapikian, A., and Bingham, R.W. 1975. Coronaviridae. Intervirology **5**: 76–82.

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