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Pigs with highly prevalent antibodies to human coronavirus and swine haemagglutinating encephalomyelitis virus in the Tohoku District of Japan

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SUMMARY

From 1985 to 1988, a total of 2496 swine sera from 60 farms in the Tohoku District of the Honshu Island of Japan were examined for antibodies to swine haemagglutinating encephalomyelitis virus (HEV), human coronavirus (HCV) and bovine coronavirus (BCV) by haemagglutination-inhibition (HI) test. Antibodies to HEV 67N strain and HCV OC43 strain were highly prevalent with positivity rates of 82·1 and 91·4%, respectively, while seropositivity rate to BCV Kakegawa strain was 44·2%. No clinical signs of HEV infection were noticed in any farms including farms with relatively high seropositivity. The results suggested that HCV or antigenitically related virus(es) as well as HEV might be perpetuated in swine in the Tohoku District.

INTRODUCTION

Coronaviruses infect a wide variety of animal species, including avians and humans, causing respiratory disease, enteritis, hepatitis, or encephalitis [1]. Transmissible gastroenteritis virus (TGEV), haemagglutinating encephalomyelitis virus (HEV) and porcine epidemic diarrhoea virus (PEDV) are known, as coronaviruses, to infect porcine species. In 1958, HEV was first reported in Canada to cause vomiting and wasting disease in young pigs and encephalitis in sucklings [2]. Since the virus was first isolated from the brain of an encephalitic pig [3, 4], many isolates of HEV have been reported [5–7]. Mortality in suckling piglets ranges from 20 to 100%, while adults are apparently uninfected [3].

Serological surveys revealed that HEV infection in swine is globally very common. In fattening pigs, seropositivities of 31% in Canada [8], 49% in England [3], 46% in North Ireland [9], 41% in Taiwan [10], 52% in Japan [11] and 0–89% in the United States [12] are reported.

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In Japan, Hirai and colleagues [11] first reported that about a half of swine sera were positive for antibodies to HEV by the haemagglutination-inhibition (HI) test, suggesting that the virus apparently exists in swine. In Japan, the virus was first isolated from the respiratory tract of piglets from two farms showing 50% or more seropositivities [13].

This paper describes the results of a serological survey for HEV, HCV and BCV in 60 swine farms of the Tohoku District.

MATERIALS AND METHODS

Serum samples

The sera collected from 2469 pigs 3–7 months of age in 60 farms in Aomori, Iwate, Akita and Miyagi prefectures from 1985 to 1988 and stored at −20 °C. These sera were heated at 56 °C for 30 min, and mixed with an equal volume of 25% (w/v) kaolin solution in Dulbecco's phosphate-buffered saline (PBS, pH 7·2) at room temperature for 30 min. After centrifugation at 8000 rpm for 10 min, the supernatants were mixed with an equal volume of 10% (v/v) chicken red blood

cell (CRBC) suspension in PBS and were incubated at room temperature for 30 min. After centrifugation at 2000 rpm for 10 min, the treated serum samples were used as 1:4 dilution.

Virus strains

Virus strains used in this test were HEV 67N strain (HEV-67N) [7], bovine coronavirus Kakegawa strain (BCV-K) [14] and human coronavirus OC43 strain (HCV-OC43) [15]. HEV-67N and BCV-K were propagated and assayed in SK-K cells [16] and BEK-1 cells [17], respectively, as reported previously. HCV-OC43 grown in mouse brain was kindly supplied by Dr R. Kawana, Department of Bacteriology, Iwate Medical University.

Mice

Specific pathogen-free ICR pregnant, and 4-week-old male mice were obtained from Japan SLC (Hamamatsu, Japan), the mice have been determined to be free from murine coronavirus infections by routine serology. The suckling mice were nursed by their dams. The suckling mice and their dams were kept in metal cages with filter caps, and 4-week-old mice were kept in plastic isolators. Animals were given autoclaved commercial pellets and water freely. The animal experiments were performed humanely in accordance with guidelines of animal experimentation of Iwate University.

Virus antigens

HEV-67N and BCV-K were inoculated into SK-K and BEK-1 cells, respectively, as previously described. Supernatants were collected after cytopathic effect was observed in the infected cell cultures, and stored at −70 °C until use. After intracerebral inoculation with HCV-OC43 into suckling mice, the brains were collected from the animals showing clinical signs of infection and were stored at −70 °C. A 10% brain homogenate (w/v) was prepared in PBS and the supernatant was used as antigen after centrifugation at 3000 rpm for 10 min.

Antisera to HEV-67N, HCV-OC43 and BCV-K

Four-week-old mice were inoculated intraperitoneally twice at an interval of 2 weeks with 0·5 ml of each virus material. At 1 week after last inoculation the mice were killed to collect the blood. The serum was heated at 56 °C for 30 min and was used as positive control. Hyperimmune guinea-pig serum against TGEV strain TO-163 had been kindly supplied by National Institute Animal Health (Tsukuba, Japan) was used to check cross reactions among the antisera and viruses.

Haemagglutination (HA) and HI tests

The tests were performed by the microtitre technique with PBS and 0·5% CRBC [11]. An HI titre of 8 or higher was recorded as positive because Kaye and colleagues [18] reported that a positive antibody response was determined on the basis of a titre of $\geqslant$ 10. The sera was diluted two-fold from 1:8 to 1:1028 in PBS and were checked for HI antibodies titres.

RESULTS

HI antibodies to HEV −67N

As shown in Table 1, 2028 out of 2469 (82·1%) sera from 4 prefectures were positive for HEV-67N, showing geometric mean titres (GMT) of 43. In Aomori Prefecture, 424 out of 493 (86%) sera were positive for HEV-67N showing the highest GMT of 74. In Iwate and Akita prefectures, 640 out of 738 (86·7%) and 337 of 530 (63·6%) sera were positive, respectively. The 26 GMT in Iwate was lowest. No clinical evidence of an HEV outbreak was found in any swine farms throughout the period of serum collection.

HI antibodies to HCV-OC43

As shown in Table 2, 2257 out of 2469 (91·4%) sera were positive for HCV-OC43 with GMT of 45 in 4 prefectures. Except for Aomori Prefecture, the sera from others showed higher positivity of more than 90%. In Miyagi Prefecture, 653 out of 708 (92·2%) sera were positive and showed the highest GMT of 58.

HI antibodies to BCV-K

HI antibodies to BCV-K were detected in 1092 out of 2469 (44·2%) sera ranging from 19·2% in Akita to 64·9% in Aomori prefectures (Table 3). The GMT varied from 12 in Iwate to 25 in Aomori prefectures. In some farms antibodies to BCV-K were undetectable.

Table 1. *Distribution of HI antibodies to HEV-67N in swine sera in four prefectures*

Prefecture (no. of farms)	Number of pigs with HI titres of								Positive/tested (%)	GMT
	< 8	8	16	32	64	128	256	512		
Aomori (14)	69	3	12	37	279	45	31	17	424/493 (86·0)	74
Iwate (16)	98	26	245	289	56	15	7	2	640/738 (86·7)	26
Akita (14)	193	45	102	111	38	19	11	11	337/530 (63·6)	29
Miyagi (16)	81	41	65	107	170	179	54	11	627/708 (82·1)	43
Total (60)	441	115	424	544	543	258	103	41	2028/2469 (82·1)	43

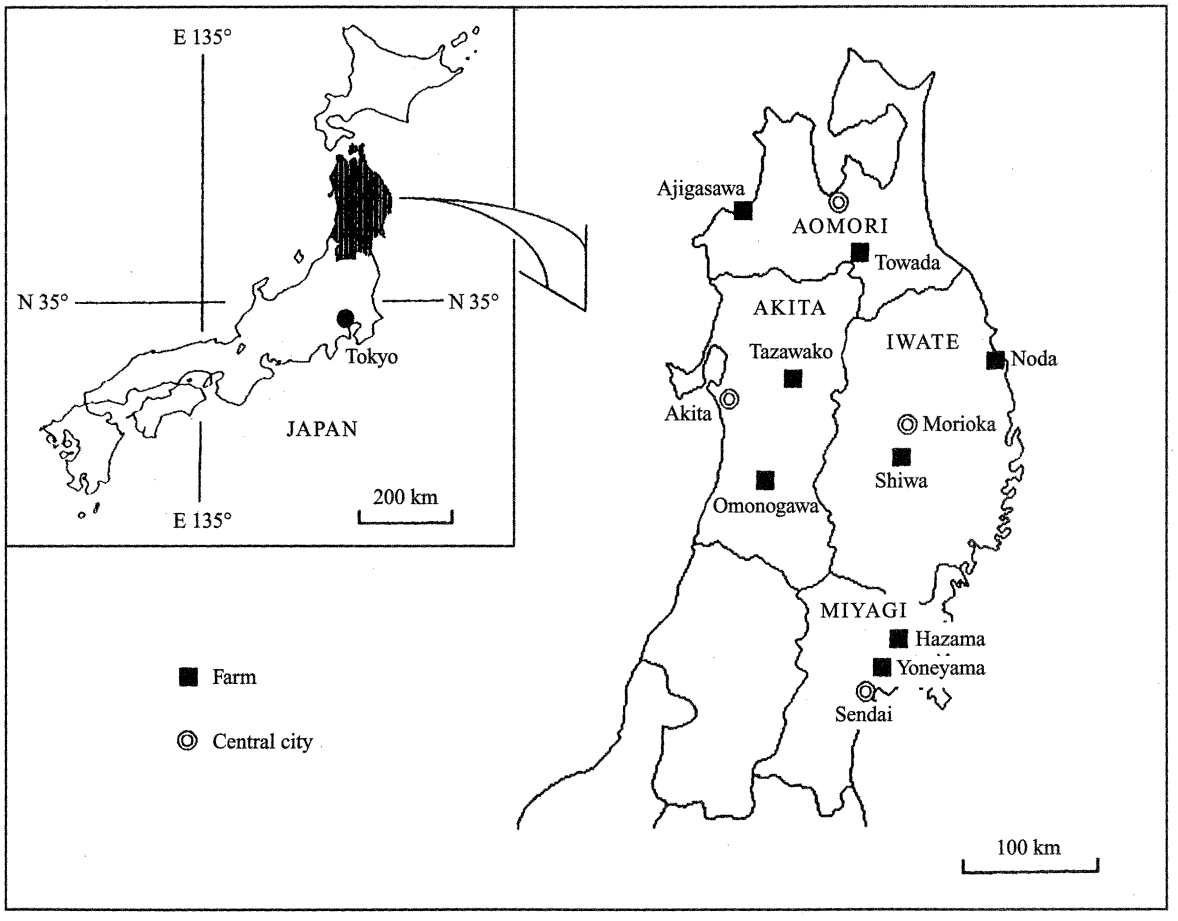

Fig. 1. Pig farms in four prefectures (Aomori, Akita, Iwate and Miyagi) in the Tohoku District of Japan.

Farms showing higher antibody titres to HEV-67N

Among 60 farms examined, those showing 100% positivity and the highest titres of HEV-67N antibody were found in each prefecture. Antibodies to HCV-OC43 and BCV-K were not higher than antibody to HEV-67N. Farm Towada (Aomori), Shiwa (Iwate), Omonogawa (Akita) and Hazama (Miyagi) showed higher antibody titres to HEV-67N than to either HCV-OC43 or BCV-K (as shown in Table 4).

Farms showing higher antibody titres to HCV-OC43

The HI antibodies to HCV-OC43 in Ajisawa (Aomori), Noda (Iwate), Tazawako (Akita) and Yomeyama (Miyagi) farms had higher positive rates

Table 2. *Distribution of HI antibodies to HCV-OC43 in swine sera in four prefectures*

Prefecture (no. of farms)	Number of sera with HI titres of								Positive/negative (%)	GMT
	< 8	8	16	32	64	128	256	512		
Aomori (14)	68	29	57	86	135	79	36	3	425/493 (86·2)	52*
Iwate (16)	57	61	137	188	172	105	15	3	681/738 (92·3)	38
Akita (14)	32	55	101	134	128	62	16	2	498/530 (94·0)	37
Miyagi (16)	55	45	63	109	230	143	58	12	653/708 (92·2)	59
Total (60)	212	190	358	517	665	382	125	20	2257/2469 (91·4)	45

Table 3. *Distribution of HI antibodies to BCV-K in swine sera in four prefectures*

Prefecture (no. of farms)	Number of sera with HI titres of						Positive/negative (%)	GMT
	< 8	8	16	32	64	128		
Aomori (14)	173	41	105	116	47	11	320/493 (64·9)	25*
Iwate (16)	480	117	112	28	1		258/783 (35·0)	13
Akita (14)	429	31	56	12	3		102/530 (19·2)	15
Miyagi (16)	296	136	134	111	27	4	412/708 (58·2)	17
Total (60)	1377	325	407	267	78	15	1092/2469 (44·2)	18

Table 4. *Swine farms showing higher antibody titres to HEV-67N*

Farm (Prefecture)	Virus (Positive/tested)	Number of sera with antibody titre of							GMT
		8	16	32	64	128	256	512	
Towada (Aomori)	HEV (73/73)	1	2	5	12	16	20	17	158*
	HCV (20/73)	2	15	2	1				17
	BCV (10/73)	1	7	2					17
Shiwa (Iwate)	HEV (27/27)	1	1	2	7	11	4	1	94
	HCV (27/27)		4	8	9	4	2		52
	BCV (20/27)	9	11						12
Omonogawa (Akita)	HEV (42/42)	1	6	8	6	10	2	9	86
	HCV (42/42)		12	12	9	3	4	2	47
	BCV (11/42)	8	2	1					10
Hazama (Miyagi)	HEV (84/84)		1	5	10	15	43	10	178
	HCV (63/84)	3	16	29	21	7	7	7	43
	BCV (32/84)	14	10	7	1				14

and antibody titres than did those to HEV-67N and BCV-K (Table 5). Especially, Farm Yoneyama showed the highest antibody titres to HCV-OC43; the GMT for HCV-OC43 was 200 in 42 swine sera. That GMT was higher than the GMT of 45 in 2257 of 2469 sera samples from other farms (Table 2), and also

Table 5. *Swine farms showing high antibody titres to HCV-OC43*

Farm (Prefecture)	Virus (Positive/tested)	Number of sera with antibody titre of 8	16	32	64	128	256	512	1028	GMT
Ajisawa (Aomori)	HEV (25/30)	4	9	8	3	1				23*
	HCV (30/30)		2	7	6	12	3			75
	BCV (13/30)	12			1					9
Noda (Iwate)	HEV (95/121)	21	37	26	8	3				20
	HCV (120/121)		11	20	37	38	14			95
	BCV (39/121)	32	6	1						10
Tazawako (Akita)	HEV (15/19)	3	5	4	3					22
	HCV (19/19)		5	6	2	1	3	2		59
	BCV (0/19)									
Yoneyama (Miyagi)	HEV (42/42)	5	7	19	6		2	3		36
	HCV (42/42)		1	1	1	13	12	11	1	200
	BCV (42/42)	9	19	10	3	1				19

Table 6. *Cross-HI test among HEV-67N, HCV-OC43, BCV-K and their antisera and anti-TGEV*

Virus	Antiserum against HEV-67N	HCV-OC43	BCV-K	TGEV
HEV-67N	1:512*	1:16	1:8	< 1:2
HCV-OC43	< 1:2	1:1024	< 1:2	< 1:2
BCV-K	1:8	< 1:2	1:1024	< 1:2

* Final serum dilution showing HI.

higher than those for HEV-67N and BCV-K. In contrast, Farm Hazama (Miyagi), located about 10 km north from Farm Yoneyama, showed the highest GMT of 178 for HEV-67N while the GMT for HCV-OC43 was 43 (as shown in Table 4).

Cross-reactivity among three viruses

To see the possibility of cross-reactivity among viruses used as HA antigen, cross-HI tests were performed. The viruses reacted with homologous antisera not with anti-TGEV guinea-pig serum. Anti-HCV-OC43 mouse serum weakly reacted with BCV-K, and mouse antiserum to BCV-K weakly reacted with HEV-67N. Anti-HCV-OC43 serum weakly reacted with HEV-67N.

DISCUSSION

In 1974, Hirai and colleagues [11] conducted the serological survey on HEV infection in pigs in Japan, revealing that about 50% of all pigs in Japan and 27% those in the Tohoku District were positive for HEV-67N. In the present study, 82% of animals in Tohoku District were shown to be positive for HEV-67N. This positivity rate was higher than those reported in Canada [8], England [6], North Ireland [9], Taiwan [10] and USA [12]. All the animals of Farm Hazama were positive for HE-67N having the highest GMT of 178. No clinical disease was observed in any of the 60 farms examined, indicating that HEV might be perpetuating as unapparent form of infection.

Hirasawa and colleague [13] isolated four strains of HEV from 5-month-old pigs with respiratory disease from two farms showing seropositivity of 90% for HEV-67N. After inoculation of the isolates into colostrum-deprived piglets, respiratory illness was produced but no vomiting.

Interestingly, HI antibodies to HCV-OC43 were detected in pigs at a higher incidence than antibodies to HEV-67N and BCV-K. Yoneyama and Hazama farms, 10 km apart from each other, showed different antibody patterns, suggesting that HCV-OC43 or antigenically related virus(es) might be responsible for HI antibodies to HCV-OC43 in Farm Yoneyama.

These findings showed that transmission of the virus among humans and pigs might occur due to close contact.

Kaye and colleagues [18] reported antigenic relationship between HCV-OC43 and HEV-67N and antibody response in human and animal sera. Both viruses have been shown to react with hyperimmune homologous sera. Sera from veterinary students and meat-packers show higher titres of HI antibodies to HEV-67N than those of college students, while swine sera appear negative for HCV-OC43 but 38% positive for HEV-67N.

The cross-HI tests suggested that antibody responses of swine sera to HEV-67N, HCV-OC43 and BCV-K might not be due to TGEV infection, and to PEDV infection because PEDV was reported to not cross-react with HEV and TGEV by Pensaert and colleagues [19] so that antibody response to HCV-OC43 might result from HCV-OC43 or antigenically related virus infection in swine.

Kaye and colleagues [20] reported the prevalence of antibodies to HCV-OC43 in children. Similarly, Kawana and Matsumoto [21] showed that 60–80% of 3- to 5-year-old children in Morioka were positive for HI antibodies to HCV-OC43, and that 6- to 9-year-old children or adults had positivities of 80–100%. These results showed that HCV was perpetuated in humans in Morioka district. These and our results also suggest the possibility that swine might become infected with HCV from unapparently infected humans.

The positivity and titres of antibodies to BCV-K were negative or much lower than titres to HCV-OC43. As described above, cross-reactivity experiments did not suggest that antibodies to BCV-K in swine might be the result of an infection with HEV or TGEV, and could be established by BCV infection after contact with cattle-keeping farmers. Because Storz and Rott [22] reported the inter-species transmission of BCV from experimentally infected calves to man, and Zhang and colleagues [23] demonstrated that a haemagglutinating coronavirus isolated from a diarrhoeic child was antigenically and genomically more closely related to BCV than to HCV-OC43.

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