A SEROLOGICAL SURVEY OF HUMAN CORONAVIRUS IN PIGS OF THE TOHOKU DISTRICT OF JAPAN

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1. ABSTRACT

A total of 2496 swine sera from 60 farms in the Tohoku District of Japan was examined for hemagglutination inhibiting (HI) antibodies to human coronavirus (HCV), swine hemagglutinating encephalomyelitis virus (HEV) and bovine coronavirus (BCV). HI antibodies to HCV OC43 strain and HEV 67N strain were highly prevalent with positivity rates of 91.4 and 82.1%, respectively, while the BCV Kakegawa strain was 44.2% positive. Farm D in Miyagi Prefecture showed the highest antibody titers to HCV OC43 strain with geometric mean titers (GMT) of 1:200. These results suggest that pigs might be infected with HCV or an antigenetically related virus as well as HEV.

2. INTRODUCTION

Coronaviruses infect a wide variety of animal species, including avian and also human beings, and causes respiratory disease, enteritis, hepatitis or encephalitis. Among the swine coronaviruses, transmissible gastroenteritis virus, porcine epidemic diarrhea virus and HEV are well known. Notably, HEV infection in pigs has been reported as globally common by serological surveys. In Japan (Hirai et al., 1974) it was first reported that about 50% of the swine sera collected were positive for HI antibodies to HEV 67N strain. In 1987 in Japan HEV was first isolated from piglets in farms showing about 50% seropositivity to the virus (Hirahara, 1987). To see the prevalence of antibodies to HEV, we undertook serological surveys for HEV in 60 farms of the Tohoku District by HI tests. In addition to HEV, we also surveyed for HCV and BCV in swine sera. The results showed that HI antibodies to HCV was higher in seropositivities and titers than those of HEV or BCV.

3. MATERIALS AND METHODS

3.1. Serum Samples

From 1985 to 1988, 2,469 sera were collected from pigs of 60 farms in Aomori, Iwate, Akita and Miyagi Prefectures. These sera were heated at 56°C for 30 minutes, and mixed with an equal volume of 25% kaolin solution in phosphate buffered saline (PBS, pH 7.2). After centrifugation, the supernatant was mixed with an equal volume of 25% chicken red blood cell (CRBC) suspension in PBS and incubated at 20°C for 30 min. After centrifugation, the serum samples were used as a 1:4 dilution of the original.

3.2. Virus

The virus strains used in this study were HCV OC436(HCV-OC43) , HEV 67N7(HEV-67N) and BCV Kakegawa (Hirano et al., 1985) (BCV-K) strains. As virus antigen, supernatant of infected mouse brain homogenates was used for HCV OC43, and infected culture fluid of SK-K for HEV 67N and of BEK-1 cells for BCV-K.

3.3. HI Test

The test was carried out in 96-well microplates using 0.5% CRBC in PBS. An HI titer of 1:8 or higher was recorded as positive.

4. RESULTS

HI Antibodies in Swine Sera to HCV-OC43, HEV-67N, and BCV-K

As shown in Table 1, 2,257 (91.4%), 2,028 (82.1%) and 1,092 out of 2,469 sera were positive for HCV-OC43, HEV-67N and BCV-K, respectively, in 4 Prefectures. The GMT for HCV-OC43, HEV-67N and BCV-K was 1:45, 1:43 and 1: 18. Among three viruses, HCV-OC43 showed the highest positive rate of 91.4% and a GMT of 1:45. The positive rates and the GMT for HCV-OC43 ranged from 86.2% (425/493) in Aomori to 94% (498/530) in Akita Prefecture, and from 1:37 in Akita to 1:59 in the Miyagi Prefecture, respectively.

Table 2 indicates swine farms showing the highest antibody titers for HCV-OC43 in each Prefecture. Farm A (Aomori), B (Iwate), C (Akita) and D (Miyagi Prefecture) had higher positive rates and antibody titers than those of HEV-67N or BCV-K. Farm D showed the highest GMT of 1:200 in 42 of 42 sera, whose antibody titers varied from 1:8 to 1:1028.

5. DISCUSSION

In 1974, Hirai et al conducted a 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 the Tohoku District were shown to be positive for HEV-67N. However, no clinical disease was observed in

Table 1. HI antibodies in swine sera to HCV-OC43, HEV-67N, and BCV-K

Virus	Prefecture	Positive/tested	(%)	GMT
HCV-OC43	Aomori	425/493	(86.2)	52 ^a
	Iwate	681/738	(92.3)	38
	Akita	498/530	(94.0)	37
	Miyagi	653/708	(92.2)	59
	Total	2257/2469	(91.4)	45
HEV-67N	Aomori	424/493	(86.0)	74
	Iwate	640/738	(86.2)	26
	Akita	337/530	(63.6)	29
	Miyagi	627/708	(82.1)	43
	Total	2028/2469	(82.1)	43
BCV-K	Aomori	320/493	(64.9)	25
	Iwate	258/783	(35.0)	13
	Akita	102/530	(19.2)	15
	Miyagi	412/708	(58.2)	17
	Total	1092/2469	(44.2)	18

^aReciprocal titer

any of the 60 farms examined. These results indicate that HEV might cause as inapparent form of infection in pigs.

Interestingly, HI antibodies to HCV-OC43 were detected in pigs at a higher incidence than antibodies to HEV-67N and BCV-K. Farm D in the Miyagi Prefecture showed the highest GMT of 1:200, suggesting that HCV or an antigenically related virus might be responsible for HI antibodies to HCV-OC43 in 42 pigs. Kaye et al reported antibody responses to HCV-OC43 and HEV-67N in human and animal sera, and demonstrated that sera from veterinary students and meat producers showed higher titers of HI antibodies to HEV-67N than those of college students, suggesting the possibility of HEV exposure in humans.

The present study demonstrated that swine sera showed high antibody titers and seropositivities for HCV-OC43 as well as HEV-67N, suggesting the possibility that HCV or antigenically related virus might infect pigs.

Table 2. Swine farms showing high antibody titers to HCV-OC43

Farm	(Prefecture)	Virus	Positive/tested (%)	GMT
A	(Aomori)	HCV-OC43	30/30 (100)	75
		HEV-67N	25/30 (83.3)	23
		BCV-K	13/30 (43.3)	9
B	(Iwate)	HCV-OC43	120/120 (100)	95
		HEV-67N	95/120 (79.2)	20
		BCV-K	39/120 (32.5)	10
C	(Akita)	HCV-OC43	19/19 (100)	59
		HEV-67N	15/19 (78.9)	22
		BCV-K	0/19	
D	(Miyagi)	HCV-OC43	42/42(100)	200
		HEV-67N	42/42(100)	36
		BCV-K	42/42(100)	19

^aReciprocal titers

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