

Prevalence of Antibodies against Transmissible Gastroenteritis Virus and Porcine Respiratory Coronavirus among Pigs in Six Regions in Japan

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ABSTRACT. A total of 2,703 pig sera from 171 farms in six regions in Japan were screened for virus-neutralizing (VN) antibody against transmissible gastroenteritis virus (TGEV). Although none of the farms had clinical signs of transmissible gastroenteritis (TGE) or vaccination against TGEV, VN antibody was detected in 14.4% of sera at 30 farms (17.5%) across all six regions. On testing of 263 VN antibody-positive sera from 27 farms with a commercial blocking ELISA to distinguish TGEV and porcine respiratory coronavirus (PRCV) antibodies, 78.3% were positive for PRCV antibody only, while 12.5% were positive for TGEV antibody only or both TGEV and PRCV antibodies. Seven of the eight TGEV antibody-positive farms were also positive for PRCV antibody. Five months after the antibody examination, a TGE outbreak occurred at one of these seven farms. These results suggest that most of the detected VN antibodies were to PRCV, and that TGEV infection might be present at some PRCV-positive farms in Japan.

KEY WORDS: antibody, Japan, PRCV, prevalence, TGEV.

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Transmissible gastroenteritis (TGE) is caused by TGE virus (TGEV), a member of the genus *Coronavirus*. On introduction into a seronegative farm, TGEV spreads rapidly to pigs of all ages, and causes severe diarrhea with high mortality, particularly in pigs aged less than 2 weeks. Economic losses from a TGE outbreak can be severe [15]. In contrast, the clinical signs of TGE are usually milder when TGEV is introduced into seropositive farms, or when TGEV infects less susceptible animals, such as sows or finisher pigs in seronegative farms. TGEV infection therefore occasionally goes undiagnosed [9, 15].

Porcine respiratory coronavirus (PRCV) is a mutant of TGEV with a characteristic large deletion (ranging from 621 to 681 nucleotides in length) in the 5' region of the spike (S) protein gene, which leads to a loss of an antigenic determinant [10, 17]. In contrast to TGEV, which replicates mainly in epithelial cells of the small intestine and causes severe diarrhea, PRCV replicates mainly in epithelial cells of the respiratory tract and causes no or mild clinical signs [15]. Since infection with PRCV induces the production of antibodies able to neutralize both TGEV and PRCV at the same titer [12], differential serological diagnosis between TGEV and PRCV infections can only be accomplished with a blocking ELISA, which uses a monoclonal antibody directed against the antigenic determinant in the S protein that is deleted in PRCV [2, 6].

The incidence and severity of TGE have decreased markedly since the widespread infection of PRCV in European swine herds in the late 1980s to early 1990s [10, 12, 13]. In the United States, a decrease in TGE incidence has been reported in one area having a high prevalence of PRCV anti-

body [20, 21]; in other TGEV- and PRCV-seronegative herds, however, TGE remains a major cause of sickness and death in piglets [14, 15].

In Japan, the incidence of epidemic TGE has decreased and only eight outbreaks were reported from 2001 to 2007 [4]. PRCV infection was first described in Japan in 1996 [8], and endemic PRCV infection was reported in pigs at a farm during a 4-year period [16]. Nevertheless, the prevalence of PRCV and TGEV infections in Japan remains obscure.

Here, to determine whether the recent decrease in reported outbreaks of TGE is due to widespread infection with PRCV, we investigated the prevalence of TGEV and PRCV antibodies in six regions in Japan using a virus neutralization test for TGEV and a blocking ELISA.

A total of 2,703 sera were collected from 171 individual swine farms (5 to 43 samples per farm) in 6 regions of Japan in 2005 or 2007, as summarized in Table 1. None of the farms had clinical signs of TGE or vaccination against TGEV. The collected sera were screened for virus-neutralizing (VN) antibody to TGEV by a virus neutralization test with the TGEV TO163 strain and CPK cells [5, 16]. VN antibody to TGEV was detected in 389 of 2,703 sera (14.4%) collected from 30 of 171 farms (17.5%) in all 6 regions. The prevalence of VN antibody to TGEV at the farm and pig levels in each region varied from 2.1% to 82.4% and from 0.9% to 53.2%, respectively. Prevalence at both the farm and pig levels was higher in the Kanto region, which has concentrated swine-rearing areas, than in other regions.

For further analysis, TGEV and PRCV antibodies were differentiated using a commercial blocking ELISA kit (SVANOVIR® TGEV/PRCV-Ab ELISA; Svanova Biotech AB, Uppsala, Sweden). This ELISA is based on the use of

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Table 1. Results of a virus neutralization test for TGEV and a blocking ELISA in pig sera in six regions in Japan

Region	Year	Virus neutralization test for TGEV	Blocking ELISA	
			TGEV antibody	PRCV antibody
Tohoku	2007	2/24 (8.3%) ^{a)} [13/240 (5.8%) ^{b)}	1/2 (50.0%) [5/13 (38.5%)]	1/2 (50.0%) [8/13 (61.5%)]
Hokuriku	2005	5/24 (20.8%) [67/709 (9.4%)]	1/5 (20.0%) [10/51 (19.6%)]	5/5 (100%) [33/51 (64.7%)]
Kanto	2005	14/17 (82.4%) [256/481 (53.2%)]	6/12 (50%) [18/157 (11.5%)]	12/12 (100%) [124/157 (79.0%)]
Chubu	2005	6/54 (11.1%) [31/330 (9.4%)]	0/6 (0%) [0/31 (0%)]	6/6 (100%) [31/31 (100%)]
Chugoku	2005	1/47 (2.1%) [8/888 (0.9%)]	0/1 (0%) [0/8 (0%)]	1/1 (100%) [8/8 (100%)]
Kyushu	2005	2/5 (40.0%) [14/55 (25.5%)]	0/1 (0%) [0/3 (0%)]	1/1 (100%) [2/3 (66.7%)]
Total		30/171 (17.5%) [389/2703 (14.4%)]	8/27 (29.6%) [33/263 (12.5%)]	26/27 (96.3%) [206/263 (78.3%)]

a) No. of positive farms /No. of tested farms (%).

b) No. of positive sera /No. of tested sera (%).

Table 2. Results of a virus neutralization test for TGEV and a blocking ELISA in pig sera in each of the TGEV antibody-positive farms

Farm	Region	Virus neutralization test for TGEV	Blocking ELISA	
			TGEV antibody	PRCV antibody
1	Tohoku	5/10 (50.0%) ^{a)}	5/5 (100%)	0/5 (0%)
3	Hokuriku	20/25 (80.0%)	10/20 (50.0%)	3/20 (15.0%)
8	Kanto	29/34 (85.3%)	3/29 (10.3%)	26/29 (89.7%)
12	Kanto	4/30 (13.3%)	1/4 (25.0%)	3/4 (75.0%)
14	Kanto	13/20 (65.0%)	4/16 (25.0%)	9/16 (56.3%)
15	Kanto	38/40 (95.0%)	2/16 (12.5%)	13/16 (81.3%)
17	Kanto	39/40 (97.5%)	1/16 (6.3%)	14/16 (87.5%)
19	Kanto	13/16 (81.3%)	7/16 (43.8%)	4/16 (25.0%)

a) No. of positive sera /No. of tested sera (%).

two monoclonal antibodies [2]: a TGEV-specific monoclonal antibody (TGEV mAb), which recognizes an antigenic determinant that is deleted in PRCV, and a monoclonal antibody, which recognizes both TGEV and PRCV (TGEV/PRCV mAb). Percent inhibition (PI) against each competing mAb was calculated according to the manufacturer's instructions. The results of ELISA were interpreted four ways, as follows. Briefly, when the test sera only blocked the binding of TGEV/PRCV mAb with a PI greater than 60%, they were considered to have antibody only to PRCV. We therefore classified them as PRCV antibody-positive sera in the present study. When they blocked the binding of both mAbs with a PI greater than 60%, they were considered to have antibody only to TGEV or antibodies to both TGEV and PRCV. We then classified them as TGEV antibody-positive sera. When they blocked the binding of both mAbs with a PI lower than 45%, they were con-

sidered to have antibody to neither TGEV nor PRCV and were determined to be negative for both TGEV and PRCV antibodies. Test sera with a PI between 45% and 60% against either or both mAbs were considered as inconclusive. The ELISA has been reported to show good agreement (κ 0.84) with the virus neutralization test for the detection of TGEV or PRCV antibody and to possess good specificity in identifying TGEV-infected herds [2].

Of the total, 263 VN antibody-positive sera collected from 27 farms, which had sufficient volume for further examination, were tested using ELISA (Table 1). Of these, 33 sera (12.5%) were TGEV antibody-positive and 206 (78.3%) were PRCV antibody-positive, while 3 and 21 were negative and inconclusive, respectively. Titers of VN antibody in the sera with negative ELISA results ranged from 1:2 to 1:8, suggesting that negative results might reflect the sensitivity of the ELISA. TGEV antibody-positive pigs

were detected in 8 farms (29.6%) in 3 regions, and PRCV antibody-positive pigs were detected in 26 farms (96.3%) in 6 regions (Table 1). Of the 27 farms, 19 farms in 6 regions had only PRCV antibody-positive pigs.

The results of the virus neutralization tests for TGEV and the blocking ELISA in each of the eight TGEV antibody-positive farms are summarized in Table 2. In seven of the eight farms, PRCV antibody-positive pigs were also detected. At farm 1, all 5 VN antibody-positive sera were TGEV antibody-positive by ELISA and there were no sera recognized as PRCV antibody-positive. Although TGEV antibody-positive pigs were identified at farms 8, 12, 14, 15 and 17, PRCV antibody-positive pigs were more predominant. On the other hand, more TGEV antibody-positive pigs were identified than PRCV antibody-positive pigs at farms 3 and 19. ELISA was positive for TGEV antibody not only in sows and gilts at farms 1, 12, 15 and 19 but also in nursery and grower-finisher pigs at farms 3, 14 and 19 (data not shown).

An epidemic TGE outbreak occurred at farm 15 five months after the antibody examination. Severe diarrhea among suckling pigs continued for 5 weeks. Morbidity in suckling pigs was nearly 100%, but mortality varied among farrowing rooms, ranging from 3.3% to 63.8%. Anorexia and diarrhea were observed in 80% and 10% of pregnant sows, respectively. Additional serum samples were therefore collected from pigs of various ages 3 and 8 months after the outbreak to observe changes in the prevalence of TGEV and PRCV antibodies. Although most of the VN antibody-positive sera were PRCV antibody-positive by ELISA at the first antibody examination (farm 15 in Table 2), at three and eight months after the outbreak, TGEV antibody-positive sera were detected predominantly in VN antibody-positive sera collected from sows, gilts and grower-finisher pigs (data not shown).

In the present study, we found that most of the detected VN antibody-positive sera were positive for PRCV antibody, and that PRCV is distributed widely in Japan, although the prevalence varies among regions. Several experimental studies have reported that PRCV can induce a variable degree of protection against TGEV infection [1, 3, 9, 18, 19]. Taken together with our data, these findings suggest that the recent decrease in epidemic TGE outbreaks in Japan may have resulted from widespread infection of PRCV, as observed in European countries [10, 12, 13]. On the other hand, in our study, TGEV antibody-positive pigs were found at several PRCV antibody-positive farms; furthermore, a TGE outbreak occurred at one of these farms, although the reason for the outbreak was unclear. A similar TGE outbreak has been reported in only one PRCV antibody-positive herd, in the United Kingdom in 1996 [7]. These results suggest that not only TGEV infection, but even TGE outbreak, can occur at a farm at which PRCV infection is present.

Further epidemiological study of TGEV and PRCV infections is needed to prevent virus transmission from farms with undiagnosed TGEV infection and to control TGE out-

breaks in Japan.

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