Short Communications

Prevalence of Coronavirus Antibodies in Iowa Swine

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ABSTRACT

Three hundred and forty-seven serum samples from 22 Iowa swine herds were screened for TGEV/PRCV neutralizing antibody. Ninety-one percent of the sera and all 22 herds were positive. These sera were then tested by the blocking ELISA test to distinguish TGEV and PRCV antibody. The ELISA test confirmed the high percentage of TGEV/PRCV positive sera. By the blocking ELISA test, 12 herds were PRCV positive, 6 herds were TGEV positive and 4 herds were mixed with sera either positive for TGEV or PRCV antibody. The results suggest a recent increase in TGEV/PRCV seroprevalence in Iowa swine most likely due to subclinical PRCV infections.

RÉSUMÉ

Des échantillons de sérum provenant de 22 troupeaux porcins de l'Iowa furent analysés pour détecter la présence d'anticorps neutralisants contre le virus de la gastro-entérite transmissible porcine (VGET) et le coronavirus respiratoire porcin (CVRP). Parmi les 347 échantillons analysés, 91% furent trouvés positifs et provenaient des 22 troupeaux. Afin de distinguer les anticorps anti-VGET des anticorps anti-CVRP, les échantillons de sérum furent testés à l'aide d'un ELISA bloquant. Cette épreuve ELISA confirma le haut pourcentage de sérums positifs. À l'aide de l'épreuve ELISA bloquant, 12 troupeaux furent identifiés comme CVRP positifs, six troupeaux comme VGET positifs et quatre troupeaux avaient des sérums positifs soit envers VGET ou CVRP. Ces résultats suggèrent une augmentation récente de la séroprévalence d'anticorps anti-VGET/CVRP dans le cheptel porcin de l'Iowa fort probablement due à des infections subcliniques causées par le CVRP.

(Traduit par docteur Serge Messier)

Transmissible gastroenteritis (TGE) is a disease of swine caused by a coronavirus that infects epithelial cells lining the small intestine. The TGE virus (TGEV) infects swine of all ages but the disease is most severe and often fatal in susceptible piglets less than 2 weeks old (1). Generally, TGE in a swine herd is recognized in either of 2 forms — epizootic or enzootic. In the epizootic (acute) form the virus sweeps through a susceptible herd and becomes self-limiting as the gestating sows and gilts acquire immunity and pass that immunity along to their litters. The herd may either free itself of the virus or become enzootically (chronically) infected if the coronavirus persists. In the enzootic form, the susceptible replacement gilts often become infected, and thus, maintain the virus in the herd.

Porcine respiratory coronavirus (PRCV) evolved from TGEV when a spontaneous deletion occurred near the N-terminus of the spike (S) gene (2). PRCV was first recognized in 1984 when a dramatic increase in the seroprevalence of TGEV antibody occurred in swine in Belgium; at that time the virus was isolated (3). The PRCV mutant virus lost its ability to infect enteric epithelial cells, and thus, no longer caused scours in pigs but did replicate in epithelial cells of the lower respiratory tract (3). PRCV spread rapidly because of subclinically infected animals and the ease of aerosol transmission so that in the next few years most swine in western Europe were infected. Later in the 1980s, a similar but slightly different deletion mutation occurred in TGEV in the United States (4). This PRCV was recognized when pigs bound for export were found to be positive for TGEV antibody (5,6). The U.S. PRCV was isolated in 1989, but since that time it has not spread as rapidly in the U.S. swine population as it did in western Europe. The 1990 National Animal Health Monitoring System (NAHMS) survey indicated that 36% of U.S. herds were positive for TGE antibody (7). That percentage was less than had been reported in 1982 for a midwest regional survey (8). The reasons for a slower dissemination of PRCV in the United States are unknown.

Two major antigenic sites on the S protein are common for both TGEV and PRCV. Thus, most serological tests cannot distinguish between TGEV and PRCV antibodies. However, due to the deletion in PRCV, a 3rd antigenic site is missing on the PRCV S protein (9). Monoclonal antibodies directed to this 3rd TGEV S protein antigenic site provide the basis of a differential blocking ELISA test that distinguishes between TGEV and PRCV antibody (10). The Diagnostic Virology Laboratory, USDA,

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TABLE I. Results of the differential blocking ELISA test and herd designation

Herd number	TGE positive	PRC positive	Negative	Designation[a]
1	15	1	0	TGE
2	0	16	0	PRC
3*	10	6	0	Mixed
4	1	15	0	PRC
5	0	16	0	PRC
6	15	0	0	TGE
7*	2	14	0	PRC
8	10	5	2	Mixed
9	0	16	0	PRC
10	0	12	4	PRC
11*	15	0	0	TGE
12*	8	8	0	Mixed
13	1	13	0	PRC
14	0	16	0	PRC
15*	1	15	0	PRC
16*	13	1	0	TGE
17	0	15	0	PRC
18*	14	1	0	TGE
19	8	8	0	Mixed
20	2	14	0	PRC
21	1	14	0	PRC
22	16	0	0	TGE

* Herds that vaccinate for TGE

[a] The predominance of either TGEV or PRCV positive sera was used to designate the herd. Nine herds had 1 or 2 sera per herd in the minority designation. This was taken as a break point to distinguish from those herds with a more equal distribution of TGEV and PRCV positive sera.

APHIS, National Veterinary Services Laboratories, Ames, Iowa, is currently providing the differential blocking ELISA test as described by Jabrane et al (11). Test results are reported to clients but the accuracy of the test is not guaranteed. This differential test is important because many swine importing countries will not accept breeding stock seropositive for TGEV antibody.

In the summer of 1995, 22 middle to large size swine producers (100 to 3000 sows per herd) participated in an Iowa State University (ISU) segregated early weaning (SEW) study (12). Sixteen pigs per producer, weaned at 10 to 21 d of age ($\bar{x}$ = 16.8 d), were transported to the ISU nursery. The pigs were bled at entry and again 39 d later, approximately 1 wk before movement to the 3rd site finishing facility. These sera were screened for TGEV/PRCV antibody to estimate the current prevalence of infection. The blocking ELISA test was carried out on the weaned pigs' entry serum samples in order to evaluate the test on a herd basis and to provide some indication on the current status of PRCV in midwestern swine.

Both sets of sera, 347 samples at entry and 335 samples at the 2nd bleeding were tested for TGEV/PRCV antibody by serum neutralization on swine testicular (ST) cells (6). Serum samples at a 1:10 dilution that neutralized virus at greater than 50% of the control value were considered positive for TGEV/PRCV antibody.

Blocking ELISA test — The blocking ELISA test was carried out with 2 monoclonal antibodies as described by Jabrane et al (11). The 1st monoclonal antibody is non-neutralizing and binds to the TGEV S protein but does not bind to the PRCV S protein. The 2nd monoclonal antibody binds to the S protein of both PRCV and TGEV (13).

Briefly, the ELISA test was carried out as follows. Microtiter plates were coated (100 µL/well) with purified TGEV antigen and incubated at 4°C. After coating, the microplates were rinsed with PBS-Tween buffer, blocked with casein and incubated at 4°C. After incubation, the blocking solution was removed and the wells were rinsed with PBS-Tween buffer. Reference sera consisted of TGEV positive, PRCV positive and negative porcine sera. Reference serum samples (100 µL/well) were added undiluted into 2 wells. The test serum samples (100 µL/well) were added undiluted into 2 wells and the microplates were incubated for 2 h at 37°C. After washes with PBS-Tween buffer, 1 of the 2 wells received 100 µL of TGEV-specific monoclonal antibody and the other well received 100 µL of TGEV/PRCV-specific monoclonal antibody. The microplates were incubated at room temperature for 30 min and washed with PBS-Tween buffer. Then, 100 µL of diluted goat anti-mouse IgG horseradish peroxidase conjugate was added to all the wells and incubated at room temperature for 30 min. After washes with PBS-Tween buffer, 100 µL of fresh tetramethylbenzidine substrate solution was added to the wells and incubated at room temperature. After 10 min, 30 µL of stop solution was added and the absorbance measured at 450 nm with a reference of 550 nm.

Sera from weaned pigs ($\bar{x}$ = 16.8 d old) upon entry to an off-site nursery were screened for TGEV/PRCV-specific antibody by a serum neutralization test. The neutralization test does not distinguish between TGEV or PRCV antibody. Ninety-one percent (316/347) of the individual pigs and all 22 herds in the study were positive for TGEV/PRCV antibody. Sixteen serum samples per herd were tested. In half of the herds (11/22), all of the pigs were positive while in the other 11 herds 9 or more pigs per herd tested positive. Seven producers vaccinated for TGE and all of these herds had strong neutralization responses at entry (109 sera tested). Thirty-nine days later, sera from a 2nd bleeding of all nursery pigs showed that 86% (287/335) of the samples were TGEV antibody positive.

A specific, monoclonal antibody based, blocking ELISA test that distinguishes between TGEV positive and PRCV positive sera was performed on the 1st set of serum samples which were obtained at entry to the nursery. This test is currently carried out by the USDA, National Veterinary Services Laboratories, Ames, Iowa, to differentiate between TGEV and PRCV positive sera. The results are summarized in Table 1. Twelve herds were PRCV positive herds. The criteria for a PRCV positive herd were that 14 or more sera were PRCV positive while 2 or less sera were TGEV positive. Two of the 12 PRCV positive herds had been vaccinated for TGE. Six herds (3 vaccinated for TGEV) were determined to be TGEV positive herds. The remaining 4 herds, 2 of which were vaccinated for

TGEV, had both TGEV and PRCV antibody positive sera (Mixed).

Two primary conclusions can be drawn from this study. First, the prevalence of TGEV/PRCV seropositive pigs in Iowa appears to be on the increase. A midwest regional study of slaughter-age pigs in 1982 showed the prevalence of TGEV antibody at 31% for individual pigs and 54% for swine herds (8). In 1990, the national NAHMS survey indicated that 36% of the swine herds in the United States were positive for TGEV antibody and that 24% of the producers surveyed vaccinated for TGE (7). Our current study, conducted during the summer 1995, showed that all of the tested 22 medium to large size Iowa swine herds were serologically positive for TGEV/PRCV antibody. This suggests that there has been a marked increase in the prevalence of TGEV antibody in midwest swine. Since replacement animals are readily transported from state to state, this increase in prevalence of TGEV/PRCV antibody might reflect changes in the swine industry nationally that have occurred since the 1990 NAHMS survey.

Second, as has been previously shown, the differential blocking ELISA test currently used by the USDA, National Veterinary Services Laboratories was effective in determining if the specific antibody was due to prior TGEV or PRCV exposure (11). For 18 of the 22 Iowa swine herds, the blocking ELISA test showed predominately either PRCV or TGEV antibody. That is, herds with 14 or more sera in the majority and only 2 sera in the minority were designated as serologically positive for the majority antibody type. Thus, the specificity of the differential test, although not absolute on an individual pig basis, still adequately designates a herd as having been exposed to either TGEV or PRCV. The remaining 4 herds were designated as herds of mixed infections because they had a large number of sera specific for both viruses.

Two herds, #7 and #15, were PRCV positive even though they were vaccinated for TGE. In 1 herd, the sows and gilts were vaccinated twice per year while in the other herd the manufacturer's recommendations were followed that gilts should be vaccinated twice before farrowing and that sows should be given a booster late in gestation. These 2 herds, like all TGE-vaccinated herds, showed a strong neutralizing response to TGEV/PRCV. If, in a single serum sample, both TGEV and PRCV antibodies are present, then blocking by the TGEV antibody should predominate and the result reported should be TGEV positive. However, this was not the case and almost all pigs from herds #7 and #15 contained only antibodies to PRCV. A possible explanation could be that high levels of PRCV antibody in the pregnant gilts and sows of herds #7 and #15 might have interfered with IM vaccination for TGE, and thus, blocked the production of TGEV antibody.

Two sets of serum samples were taken during this study: at entry to the nursery and prior to entering the finishing facility. The entry samples were chosen for use in the blocking ELISA test because they measure passive antibody and early exposure, and thus, indicate infections that had occurred in each herd. Pigs upon arrival at the ISU nursery were commingled by weight and by herd of origin. No differential test was carried out on the 2nd set of samples because this would only measure the results of commingling at the nursery site. The serum neutralization screening test was carried out on the second set of serum samples and did not indicate an increase in TGEV/PRCV-specific antibody while in the nursery.

In 1984, PRCV was first recognized in Belgium by a sudden increase in the prevalence of TGEV antibody (68% of the herds in the survey were positive for TGEV antibody). Later that year, a spontaneous mutant of TGEV was isolated and the new virus, termed PRCV, quickly spread because of the respiratory nature of the infection. It became endemic in all western European countries in about a two-year time period. The rapid dissemination of PRCV was not accompanied by a dramatic increase in the severity of respiratory disease. Instead PRCV infections were subclinical and generally beneficial by providing partial immunity to TGE which then declined (2). In the United States, a PRCV phenotypically similar to the European PRCV, was isolated in Indiana, North Carolina, and Minnesota after a serologic screen for TGE antibody. However, the spread of PRCV in the United States has been curtailed, perhaps because of the awareness of the rapid PRCV spread in western Europe and the availability to test for TGEV antibody. Indeed, results from the 1990 NAHMS survey indicated no dramatic increase in the prevalence of TGEV antibody during the decade of the 1980's. However, since 1990, there has been an expressed desire by veterinary practitioners for the USDA, National Veterinary Services Laboratories to conduct a reliable TGEV/PRCV differential test. The results from our limited study in 1995 suggest that many farms in Iowa have been unknowingly exposed to PRCV. Based on the relatively clear distinction of antibody amongst herds, the Canadian blocking ELISA test effectively differentiated herds exposed to either PRCV or TGEV.

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