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Field validation of a commercial blocking ELISA to differentiate antibody to transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus and to identify TGEV-infected swine herds

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Abstract. A commercially available blocking ELISA was analyzed for its ability to identify antibodies to porcine coronaviruses (transmissible gastroenteritis virus [TGEV] or porcine respiratory coronavirus [PRCV]), to differentiate antibodies to TGEV and PRCV, and to identify TGEV-infected herds. Nine sera from uninfected pigs, 34 sera from 16 pigs experimentally infected with TGEV, and sera from 10 pigs experimentally infected with PRCV were evaluated using both the TGEV/PRCV blocking ELISA and a virus neutralization (VN) assay. The ELISA was not consistently effective in identifying pigs experimentally infected with TGEV until 21 days postinfection. Sera from 100 commercial swine herds (1,783 sera; median 15 per herd) were similarly evaluated using both tests. Thirty of these commercial herds had a clinical history of TGEV infection and a positive TGEV fluorescent antibody test recorded at necropsy within the last 35 months, while 70 herds had no history of clinical TGEV infection. The blocking ELISA and the VN showed good agreement (kappa 0.84) for the detection of porcine coronavirus antibody (TGEV or PRCV). The sensitivity (0.933) of the ELISA to identify TGEV-infected herds was good when considered on a herd basis. The ELISA was also highly specific (0.943) for the detection of TGEV-infected herds when the test results were evaluated on a herd basis. When sera from specific age groups were compared, the ELISA identified a greater proportion (0.83) of pigs in herds with TGEV antibody when suckling piglets were used. In repeatability experiments, the ELISA gave consistent results when the same sera were evaluated on different days (kappa 0.889) and when sera were evaluated before and after heating (kappa 0.888). The blocking ELISA was determined to be useful for herd monitoring programs and could be used alone without parallel use of the VN assay for the assessment of large swine populations for the detection of TGEV-infected herds.

Transmissible gastroenteritis (TGE) is a highly contagious enteric disease of swine. During an outbreak of TGE in a naive swine herd, 100% of infected piglets less than 2 weeks of age may experience vomiting, diarrhea, dehydration, and death. However, in herds with endemic TGE, the majority of piglets suckling dams providing milk antibody are unaffected,[14] making it difficult to identify these endemically infected herds clinically. It is important to identify these TGE virus (TGEV)-infected swine herds serologically to prevent spread of the disease to TGEV-negative herds by the introduction of infected animals. Until recently, the virus neutralization (VN) assay was used to evaluate antibody to TGEV. However, porcine respiratory coronavirus (PRCV), which causes mild respiratory infection,[11] has now become serologically evident in swine in Ontario. Antibody to TGEV and PRCV cannot be distinguished using the VN assay.[3,7] Therefore, a different test is now required to identify TGEV-infected herds in large swine populations.

Monoclonal antibodies to epitopic differences on the S protein can differentiate TGEV and PRCV.[3] Numerous serological ELISAs based on these epitopic differences and monoclonal antibodies have been developed to differentiate antibodies to TGEV and PRCV.[2,4,8,15,17] However, few have been validated using large numbers of field sera collected from commercial herds[18] or are commercially available for use in diagnostic laboratories.

The purpose of this study was to evaluate a commercially available TGEV/PRCV blocking ELISA[a] for identification of TGEV-infected swine herds. The objectives of the study were to determine the sensitivity of the TGEV/PRCV ELISA for the identification of TGEV-infected commercial swine herds, to determine the specificity of the TGEV/PRCV ELISA to identify TGEV-negative commercial swine herds, to determine if this ELISA can be used alone or if the VN assay should be used in parallel with the TGEV/PRCV ELISA, and to determine if the TGEV/PRCV ELISA could be used for individual animal testing or should be used for herd-based testing.

Materials and methods

Sera collected from experimentally infected pigs. Sera were obtained from 9 pigs free from antibody to all coro-

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Table 1. Comparison of the transmissible gastroenteritis virus/porcine respiratory coronavirus (TGEV/PRCV) blocking ELISA, with percent inhibition for each competing monoclonal antibody and porcine coronavirus virus neutralization (VN) titers for uninfected pigs and pigs experimentally infected with either TGEV or PRCV.

Pig	Day post-infection	Infecting virus dose ($CCID_{50}$)	TGEV/PRCV ELISA result	% inhibition anti-TGEV monoclonal antibody	% inhibition anti-TGEV/PRCV monoclonal antibody	VN titer
Sera from uninfected pigs from Iowa State University						
10	0	none	neg	15.55	8.02	<1:8
11	0	none	neg	17.72	11.06	<1:8
12	0	none	neg	16.12	16.50	<1:8
Sera from uninfected pigs and following oral infection with Miller #5B Strain TGEV at National Veterinary Services Laboratory						
401	0	none	neg	7.4	10.97	<1:8
	7	6×10^5	neg	33.11	35.91	<1:8
	11		PRCV*	34.90	51.61	<1:8
	14		TGEV†	62.40	62.04	<1:8
	21		TGEV	80.58	77.01	1:64
402	0	none	neg	8.64	4.73	<1:8
	7	2×10^5	neg	29.63	32.47	<1:8
	11		neg	31.31	47.74	<1:8
	14		PRCV	48.26	66.12	1:16
	21		TGEV	57.80	69.57	1:64
403	0	none	neg	17.73	00.00	<1:8
	7	6×10^4	neg	36.70	26.00	<1:8
	11		neg	22.90	49.03	<1:8
	14		PRCV	35.91	63.33	<1:8
	21		TGEV	65.77	82.47	1:128
404	0	none	neg	18.41	5.91	<1:8
	7	2×10^4	neg	31.20	38.28	<1:8
	11		neg	33.56	45.81	<1:8
	14		TGEV	60.83	61.94	1:64
	21		TGEV	75.87	72.47	1:128
405	0	none	neg	8.42	5.29	<1:8
	7	6×10^3	neg	26.15	36.13	<1:8
	11		neg	24.92	42.69	<1:8
	14		TGEV	61.39	66.02	1:32
	21		TGEV	67.68	76.99	1:16
406	0	none	neg	14.6	7.42	<1:8
	7	2×10^3	neg	23.34	34.30	<1:8
	11		PRCV	30.98	51.18	<1:8
	14		PRCV	39.28	59.35	1:128
	21		TGEV	61.62	75.81	1:16
407	9	2×10^6	neg	19.98	47.42	1:16
408	9	2×10^6	neg	40.00	53.55	1:32
409	11	2×10^6	PRCV	24.35	50.43	1:16
410	14	2×10^6	neg	29.07	43.76	<1:8
Sera from pigs infected orally with Miller strain TGEV at Iowa State University using 2 ml of a suspension of untitered intestinal content						
76	35	NT‡	TGEV	81.35	87.82	1:128
77	35	NT	TGEV	66.37	88.98	1:96
78	35	NT	TGEV	84.83	89.56	1:768
88	35	NT	TGEV	86.81	87.46	1:128
89	35	NT	TGEV	88.13	91.59	1:512
90	35	NT	TGEV	80.41	90.14	1:48
Sera from pigs infected with 4 different strains of PRCV at the National Veterinary Services Laboratory						
223	37	NA§	PRCV	42.87	82.47	>1:512
243	37	NA	PRCV	40.52	83.23	>1:512
247	40	NA	PRCV	32.66	76.99	1:256
278	37	NA	PRCV	26.94	82.47	>1:512

Table 1. Continued.

Pig	Day post-infection	Infecting virus dose ($CCID_{50}$)	TGEV/PRCV ELISA result	% inhibition anti-TGEV monoclonal antibody	% inhibition anti-TGEV/PRCV monoclonal antibody	VN titer
Sera from pigs inoculated orally and intranasally with PRCV Strain 5170 at Iowa State University						
22	35	6×10^2	PRCV	32.80	88.97	1:256
23	35	6×10^2	TGEV	59.02	89.42	1:1,536
23	(retest)		PRCV	44.10	84.33	
24	35	6×10^2	PRCV	48.19	91.37	1:256
34	35	6×10^2	PRCV	43.10	86.66	1:768
35	35	6×10^2	PRCV	34.81	88.84	1:96
36	35	6×10^2	PRCV	34.06	88.98	1:128

* PRCV = PRCV ELISA positive.
† TGEV = TGEV ELISA positive.
‡ NT = suspension not titrated.
§ NA = information on virus dose not available.

naviruses (9 sera), from 16 pigs experimentally infected with TGEV (34 sera), and from 10 pigs experimentally infected with PRCV (10 sera) (Table 1) to determine the sensitivity and specificity of the ELISA to detect TGEV-infected pigs and PRCV-infected pigs, respectively, and to determine the level of agreement of the ELISA with the VN assay for the detection of porcine coronavirus antibody to TGEV or PRCV.

Three experimental sera without antibody to porcine coronaviruses came from Iowa State University (ISU), while 6 came from the National Veterinary Services Laboratory (NVSL), Ames, Iowa.

Sera with antibody to TGEV came from 3 separate TGEV challenge experiments where pigs were infected with the Miller #5B strain of TGEV. Pigs 401–406 were infected orally at 28 days of age with 2×10^3 $CCID_{50}$ to 6×10^5 $CCID_{50}$ and bled sequentially (NVSL) (experiment 1). Pigs 407–410 were orally infected with 2×10^6 $CCID_{50}$ at 40 days of age and bled once at day 9, 11, or 14 (NVSL) post-infection (experiment 2). Pigs 76–78 and 88–90 were experimentally infected orally with a 2-ml suspension of untitrated intestinal contents from an infected pig (ISU) and bled once at 35 days (experiment 3).

Similarly, sera from PRCV experimentally infected pigs came from 2 separate PRCV challenge experiments. Pigs 223, 243, 247, and 278 each received a different strain of PRCV (NVSL) and were bled at day 37 or 40 (experiment 1). Pigs 22–24 and 34–36 each received 3×10^2 $CCID_{50}$ orally and 3×10^2 $CCID_{50}$ intranasally of PRCV strain 5170 (ISU) and were bled on day 35 (experiment 2).

Sera collected from 100 commercial swineherds. A total of 1,783 sera (median of 15 sera per herd) (Table 2) were collected from individual pigs of various ages (Table 2) from each of 100 swine herds.

Thirty of these herds had a previous clinical history of TGE (Table 2). Piglets from these 30 TGEV-positive herds also had a positive TGEV fluorescent antibody (FA) test recorded at necropsy within the last 2 years except for 1 TGE-infected herd where the last positive FA test was recorded 35 months prior to sera collection. These herds were located in Ontario, Quebec, Manitoba, Alberta, and North Carolina.

The remaining 70 swine herds (Table 2) were considered not to be infected with TGEV. These herds were identified by attending veterinarians as being free of the clinical signs of TGE. Herds were located in Ontario, Quebec, Manitoba, and Alberta.

Sera were collected from boars, sows, sows or gilts, grow-finish, nursing, and suckling pigs. The age of pig was not given for some sera.

Sera collected from specific age groups to determine the prevalence of antibody within age groups. Sera were specifically collected from 4 different age groups: sows, grow-finish (50–180 days), nursery (21–49 days), and suckling (birth to 20 days) pigs, with 15 sera collected from each age group from each of 3 herd types (1 herd with no porcine coronavirus antibody, 1 herd with antibody only to PRCV, and 1 herd with antibody to both TGEV and PRCV, as determined by the ELISA). These sera were tested using both the ELISA and the VN test.

Sera for repeatability and effect of heating. To determine the repeatability of the ELISA, 60 field sera collected from 3 TGEV clinically infected herds were tested twice on different days. Sera were refrigerated between test days. In addition, to evaluate the effect of heating at 56 C for 30 min, these same 60 sera were tested after heating. These sera were also tested using the VN assay to assess agreement between the 2 tests for the detection of antibody to porcine coronaviruses (TGEV or PRCV).

TGEV/PRCV ELISA. Except as noted above, all sera were evaluated unheated using the TGEV/PRCV blocking ELISA according to the instructions supplied in the kit. Briefly, sera were diluted 2-fold and added in duplicate to individual rows of wells of microtiter plates coated with TGEV antigen. Swine anti-TGEV or anti-PRCV antibodies, if present in the test samples, were bound to the TGEV antigen on the plate and blocked the subsequent binding of either mouse IgG anti-TGEV and/or anti-TGEV/PRCV monoclonal antibodies that were added to the respective row of wells. The addition

Table 2. Summary of population demographics in transmissible gastroenteritis (TGE) clinically positive and negative herds with numbers in each group and proportion positive.

Age	TGE-positive herds*	TGE-negative herds†	Totals‡
Herds	30	70	100
Median number of animals	15	15	
Boars	4 (0.25)	17 (1.0)	21 (0.01)
Sows	152 (0.76)	156 (1.0)	308 (0.17)
Sows/gilts	49 (0.78)	45 (1.0)	94 (0.05)
Gilts	30 (0.23)	75 (1.0)	105 (0.06)
Grower-finish	75 (0.13)	219 (1.0)	294 (0.16)
Nursery	109 (0.66)	281 (0.96)	390 (0.22)
Suckling	94 (0.83)	39 (1.0)	133 (0.08)
Age not given	126 (0.71)	312 (1.0)	438 (0.25)
Total animals	639	1,144	1,783

* Proportion testing TGE ELISA positive in TGE clinically infected herds given in parentheses.

† Proportion testing TGE ELISA negative in TGE clinically negative herds given in parentheses.

‡ Proportion of all 1,783 pigs tested given in parentheses.

of horseradish peroxidase conjugated antimouse IgG antibodies resulted in a colorless reaction where antibodies were present in the test sera. Alternatively, if there were no specific coronavirus antibodies in the test sera, the monoclonal antibodies bound to the plate so there was a strong color change in both wells. The optical density of this color change was measured at 450 nm. The optical density of the test samples was compared with the optical density of the negative control included in the kit to determine a cut-off value (0.5 × mean optical density of the negative control antisera) using each monoclonal antibody. Because this is a blocking assay, sera with optical densities greater than the cut-off values were considered negative. Only 3 interpretations for results were possible. Where swine sera were able to block both anti-TGEV and anti-TGEV/PRCV monoclonal antibodies, the sera were considered to have antibodies to TGEV. When swine sera were able to block only the anti-TGEV/PRCV monoclonal antibody, the sera were determined to have antibodies only to PRCV. When sera blocked neither monoclonal antibody, the sera were determined to be negative for antibodies to both porcine coronaviruses. For sera from all experimentally infected pigs (Table 1) and 13 selected sera from 4 herds without clinical history of TGE, the percent inhibition was also calculated and reported for each competing monoclonal antibody (% inhibition = 100 − [sample optical density × 100/mean optical density negative control]). Percent inhibitions greater than 50% imply the presence of competing antibody in test sera and can be used as an indicator of level of antibody.

Virus neutralization. After ELISA testing was complete, sera were subsequently heated at 56 C for 30 min and evaluated for virus neutralizing (VN) antibody to porcine coronaviruses using a standard microtiter VN assay. Serial 2-fold dilutions of sera were made in 96-well microtiter plates. Approximately 100 $CCID_{50}$ of Diamond strain TGEV was added to each well. After 1 hr of incubation at 4 C, swine testicle (ST) cells were added and the plates were incubated at 37 C in a 5% CO_2 atmosphere for 3–4 days. Each well was then examined for cytopathic effect. The antibody titer was determined to be the dilution of sera where 50% of the wells were infected. Known positive and negative TGEV antisera were included in each assay. Sera with titers <1:8 were considered negative for antibody to porcine coronaviruses. Sera with titers above this value were presumed to have antibody to porcine coronaviruses. Because the VN test readily identifies antibody to both porcine coronaviruses, it cannot distinguish between antibody to TGEV or PRCV.

Statistical methods. Standard statistical methods were used to determine the sensitivity,[13] specificity,[13] multirater kappa,[16] McNemar's chi-square test,[12] and sensitivity covariance[6] for data presented for the evaluation of the ELISA and its comparison to the VN assay. Kappa is the proportion of potential agreement achieved beyond chance.[16] The McNemar's chi-square test compares 2 populations in paired data.[12] Determining the sensitivity covariance is a relatively new technique that is recommended to estimate the conditional dependence between diagnostic tests used in combination.[6] Tests used in parallel or series are usually assumed to be conditionally independent, and the resultant sensitivity of the tests used in combination are calculated directly from the individual test values.[6] This can overinflate the true sensitivity or specificity of the combined tests. When tests measure similar analytes such as serum antibodies, the test results are likely to be dependent. Knowing the test covariances will aid the user in determining if more tests will increase the certainty of the diagnosis.[6] Exact binomial 95% confidence intervals used throughout this article were calculated using epidemiologic software.[b] Statistical significance is designated by a probability (P) value <0.05.

Clinical status, as previously described, was used as the gold standard for herd classification. For herd-level testing, if one animal tested positive for antibody to TGEV, the herd was considered positive for TGEV. Herd-level sensitivity is the proportion of infected herds that have TGEV ELISA reactors greater than or equal to the cutpoint of 1 positive animal. Herd-level specificity is the proportion of noninfected herds that have TGEV ELISA reactors less than the cutpoint.

Since prevalence in infected herds was not known and a gold-standard test for TGEV-specific antibody in individual animals is not available, clinical status could not be used as the gold standard for individual animals. Individual animal sensitivity and specificity could therefore not be determined. Instead, the relative sensitivity and relative specificity of the VN test and blocking ELISA were determined.

Using the above parameters, the ELISA and the VN were initially compared for their ability to identify antibody to porcine coronaviruses (TGEV or PRCV) using 34 sera collected from 16 animals experimentally infected with TGEV and 10 animals experimentally infected with PRCV. Sera from 9 uninfected control animals were also evaluated. These sera were also used to determine the sensitivity and specificity of the ELISA to identify TGEV-infected and PRCV-infected pigs. Because the control group of sera from uninfected pigs consisted mostly of sera collected from pigs at day 0 prior to infection, a control group was not available

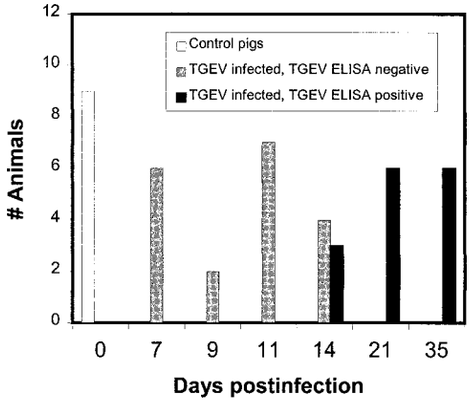

Figure 1. Transmissible gastroenteritis virus (TGEV)/porcine respiratory coronavirus (PRCV) ELISA results following experimental TGEV infection for days postinfection.

to compare the ELISA and VN results for sera collected on individual days postinfection. Therefore, all sera collected from 7 to 35 days postinfection were used as a composite to calculate sensitivity and specificity for the identification of porcine coronavirus antibody (TGEV and PRCV) and antibody specific for TGEV and PRCV.

Subsequently, the ELISA and VN tests were compared for their ability to identify antibody to porcine coronaviruses (TGEV or PRCV) in individual animals using the 1,783 (median 15 per herd) sera collected from the 100 commercial swine herds. Next, the ELISA was evaluated statistically for its ability to detect the 30 TGEV clinically positive and 70 TGEV clinically negative herds. The effect of specific age groups of the pig, the repeatability of the assay, and the effect of heating of the sera were also statistically evaluated.

Results

Analysis of testing for sera collected from experimentally infected pigs. The TGEV/PRCV ELISA result, the percent inhibition for both the anti-TGEV and the anti-TGEV/PRCV competing monoclonal antibodies, and VN antibody titers for the 53 sera collected from 29 experimental pigs are presented in Table 1. All 9 sera from uninfected pigs were negative for antibody to both porcine coronaviruses in both the ELISA and VN tests. For the 10 pigs experimentally infected with TGEV, where sera were collected early after infection, the ELISA determined sera to be either negative for antibody to both porcine coronaviruses or to have PRCV antibody. The ELISA did not consistently determine the TGEV status of TGEV-infected pigs correctly until 21 days postinfection (Fig. 1), when all 6 sequentially bled animals were determined to have antibody to TGEV using the ELISA (percent inhibition for both blocking monoclonal antibodies >50%). Antibody to porcine coronaviruses was similarly not consistently demonstrated until 21 days postinfection using the VN test. For the 10 pigs experimentally infected with PRCV, the ELISA correctly declared 9 to be antibody positive for PRCV (>50% inhibition for only anti-TGEV/PRCV monoclonal antibody) but incorrectly affirmed 1 to be positive for antibody to TGEV (Table 1, pig 23), with >50% inhibition for both anti-TGEV (59%) and anti-TGEV/PRCV (89%) monoclonal antibodies. When this serum was retested, it was determined to have only antibody to PRCV, with percent inhibition to the anti-TGEV monoclonal antibody being only 44% on the second test.

When all sera collected from experimentally infected pigs at various days postinfection were evaluated, the ELISA (Table 3) showed an overall low sensitivity (0.441) for the detection of TGEV infection. After 21 days postinfection, all pigs were TGEV ELISA positive, compatible with 100% sensitivity. The VN test (Table 3) also demonstrated low sensitivity (0.559) for the detection of animals with porcine coronavirus antibodies (TGEV or PRCV). A McNemar's chi-square test did not detect significant differences ($P > 0.05$) between sensitivities of the VN and ELISA to detect porcine coronavirus antibody (antibody to TGEV or PRCV). Both tests were highly specific (1.0) for pigs not infected with TGEV.

Parallel use of the VN test slightly improved the detection of animals with antibody to TGEV (0.535) and for porcine coronaviruses (0.623) (Table 4), al-

Table 3. Overall sensitivity and specificity for detection of antibody specific to transmissible gastroenteritis virus (TGEV) using the TGEV/porcine respiratory coronavirus (PRCV) blocking ELISA and for antibody to porcine coronaviruses (TGEV or PRCV) using the virus neutralization (VN) assay, including 34 sera from all animals experimentally infected with TGEV collected 7–35 days postinfection and 9 uninfected control sera.

Test	Test results	TGEV infected	Uninfected controls	Sensitivity*	Specificity
ELISA	TGEV positive	15	0	0.441 (0.272, 0.621)†	1.0 (0.664, 1.0)†
	TGEV negative	19	9		
VN	Positive	19	0	0.559 (0.379, 0.728)†	1.0 (0.664, 1.0)†
	Negative	15	9		

* No significant difference in sensitivities of TGEV ELISA and VN tests using McNemar's chi-square test.
† Exact binomial 95% confidence interval given in parentheses.

Table 4. Parallel sensitivity, test covariances, and kappa values for the transmissible gastroenteritis virus (TGEV) or porcine respiratory coronavirus (PRCV) ELISA and the virus neutralization (VN) for detection of antibody to TGEV or antibody to porcine coronaviruses (TGEV or PRCV) in sera collected from pigs experimentally infected with TGEV from 7–35 days postinfection.

Virus used for experimental infection	Parallel sensitivity with VN	Sensitivity covariance	Sensitivity covariance *P*-values	Kappa
TGEV (n = 43)	0.535 (0.377, 0.678)*	0.21	<0.0005†	0.82
PRCV or TGEV (n = 53)	0.623 (0.479, 0.752)*	0.21	<0.0005†	0.83

* Exact binomial 95% confidence interval given in parentheses.
† Chi-square test (Med-Calc).

though the gain in sensitivity was offset by the significant covariance (<0.0005) between the 2 tests, indicating that the tests were conditionally dependent.[6]

The level of agreement between the ELISA and the VN test for detection of sera with antibody to coronaviruses (TGEV or PRCV) for TGEV experimentally infected pigs was high (kappa = 0.82) (Table 4). The level of agreement between the ELISA and VN to identify pigs experimentally infected with either TGEV or PRCV was also high (kappa = 0.83) (Table 4), with relative sensitivity = 1.0 and relative specificity = 0.83 (data not shown).

Analysis of testing for sera collected from 100 commercial swine herds. A total of 1,783 sera from 100 commercial swine herds were evaluated using both the ELISA and the VN. Of the 30 herds with a clinical history of TGE and a positive TGEV FA test, all but 2 herds had at least 1 animal with antibodies specific to TGEV detected by ELISA. Of special note is that 17 of these 30 herds had pigs that tested positive for ELISA antibody to PRCV in addition to pigs that tested ELISA positive for antibody to TGEV. A bar graph shows the relationship between the ELISA result and the VN antibody titer in TGEV clinically positive herds (Fig. 2). The PRCV positive ELISA results in the 30 TGEV clinically positive commercial herds were more commonly associated with VN titers less than 1:128, although this did not appear to be the case for experimentally infected pigs. However, similar to experimentally infected pigs, pigs with low VN titers in TGEV-infected herds were more likely to have a positive PRCV ELISA. The results for 1 serum were inconclusive using the ELISA.

For the 70 herds presumed to be free from clinical signs of TGE, 43 herds were free from antibody to both porcine coronaviruses, using both the ELISA and the VN test, while 23 herds were determined to have antibody only to PRCV using the ELISA. Four herds believed to be free of TGE had at least 1 pig with TGEV ELISA antibody (1/15, 1/15, 3/15, 8/15). For these 4 herds, the percent inhibition for the anti-TGEV monoclonal antibody ranged from 52.15 to 90.37%, while the percent inhibition for the anti-TGEV/PRCV monoclonal antibody ranged from 75.21 to 95.62%. The VN titers ranged from 1:6 to >1:4,096.

When all 1,783 sera from commercial herds were included in a statistical analysis (Table 5) to identify porcine coronavirus antibody (TGEV or PRCV) and were assessed on an individual animal basis, the ELISA and the VN assay showed good agreement, with kappa = 0.84 (relative sensitivity = 0.979; relative specificity = 0.964).

When the statistical evaluation was considered on a herd basis (Table 6), where a herd was considered

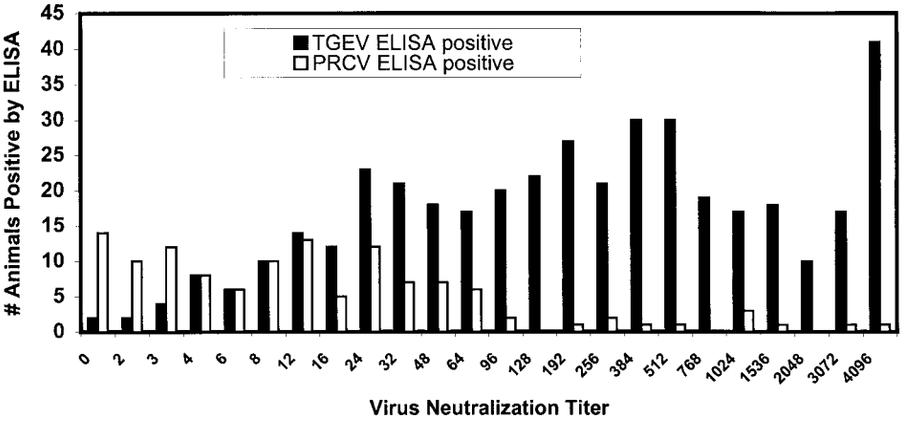

Figure 2. Relationship of transmissible gastroenteritis virus (TGEV)/porcine respiratory coronavirus (PRCV) ELISA to virus neutralization (VN) titers in TGEV clinically positive herds.

Table 5. Comparison of relative sensitivity, specificity and agreement of the transmissible gastroenteritis virus (TGEV)/porcine respiratory coronavirus (PRCV) ELISA and virus neutralization (VN) assay to identify porcine coronavirus antibody (TGEV or PRCV) in 1,783 sera form 100 commercial swine herds on an individual animal basis.

ELISA result	VN positive	VN negative	Relative sensitivity	Relative specificity	Kappa
TGEV ELISA or PRCV ELISA positive	774	36	0.979 (0.966, 0.987)*	0.964 (0.950, 0.974)*	0.84
TGEV ELISA or PRCV ELISA negative	17	955			

* Exact binomial 95% confidence interval given in parentheses.

ELISA positive for TGEV if the serum from 1 animal had a positive TGEV ELISA, the sensitivity of the ELISA to detect TGEV-positive herds was good (sensitivity = 0.933). The herd-level specificity (0.943) to detect TGEV-negative herds was also good.

When age was considered for all 1,783 pigs from the 100 herds (Table 2), the ELISA was most effective in identifying antibody to TGEV when suckling pigs (proportion tested positive = 0.83) were evaluated. This is in contrast with grow-finish pigs, where only 13% tested positive for antibody to TGEV.

Analysis of testing for sera collected from specific age groups. When 15 animals from each of 4 age groups were specifically bled from 3 selected herds (1 herd free from clinical TGE and without coronavirus antibody [TGEV or PRCV] by ELISA, 1 herd without history of clinical TGE but with ELISA antibody to PRCV, and one herd with a history of clinical TGE and pigs with ELISA antibody to TGEV and other pigs with ELISA antibody to PRCV), more sows and nursing and suckling piglets consistently had antibody to coronaviruses (80–100%) when compared with grow-finish pigs (40%) (Fig. 3).

Discussion

When 60 sera from herds with a history of clinical TGE were evaluated on a second day, the ELISA showed good repeatability (kappa = 0.889). Heat inactivation (56 C for 30 minutes) of the same 60 sera did not adversely affect the ELISA (kappa = 0.888).

Analysis of sera for repeatability and effect of heating. When all sera collected from pigs experimentally infected with TGEV at various times postinfection were evaluated, the commercial ELISA had low sensitivity (0.441) for the detection of TGEV infection. Since this is a blocking assay, the ELISA would have detected both swine IgM and IgG antibody. Therefore, the delay in identification of antibody is most likely due to the effect of days postinfection and time for pigs to mount an antibody response to the specific viral epitopes targeted by the murine monoclonal antibodies. For experimentally infected pigs, the sensitivity of the ELISA to identify TGEV-infected pigs was 100% only at 21 days postinfection. This indicates limitations for the ELISA for the early serological diagnosis of acute disease outbreaks and would be an important consideration for application of the ELISA in herd testing programs.

However, the sensitivity (0.933) of the ELISA to identify TGEV-infected commercial swine herds was good when the ELISA was evaluated on a herd basis, where a herd was considered positive if 1 sera from the herd was TGEV ELISA positive. The ELISA was also highly specific (0.943) when the analysis was performed on a herd basis. The ELISA identified TGEV antibody in 4 commercial herds that were reported to be free from clinical TGEV infection. Further investigations to determine the reasons for these results in these 4 herds were not possible. The specificity of the test would be increased by increasing the cutoff of test-positive animals to more than 1. However, that would also result in a decrease in herd-level sensitivity.

The variability in the proportion of animals among different age groups that are positive for TGEV-specific antibody would be important when considering the age of pig to bleed and/or the number of samples required for effective herd monitoring.[5] For herd monitoring, this study suggests the ELISA would be most sensitive and would require fewer animals to be tested when suckling piglets with maternal antibody are used

Table 6. Comparison of the sensitivity and specificity of the transmissible gastroenteritis virus (TGEV)/porcine respiratory coronavirus (PRCV) ELISA to detect TGEV clinically positive herds. A herd was diagnosed as positive for TGEV if 1 animal was positive for antibody to TGEV.

ELISA result	TGEV herds clinically positive	TGEV herds clinically negative	Sensitivity	Specificity
TGEV ELISA positive	28	4	0.933 (0.779, 0.992)*	0.943 (0.86, 0.984)*
TGEV ELISA negative	2	66		

* Exact binomial 95% confidence interval given in parentheses.

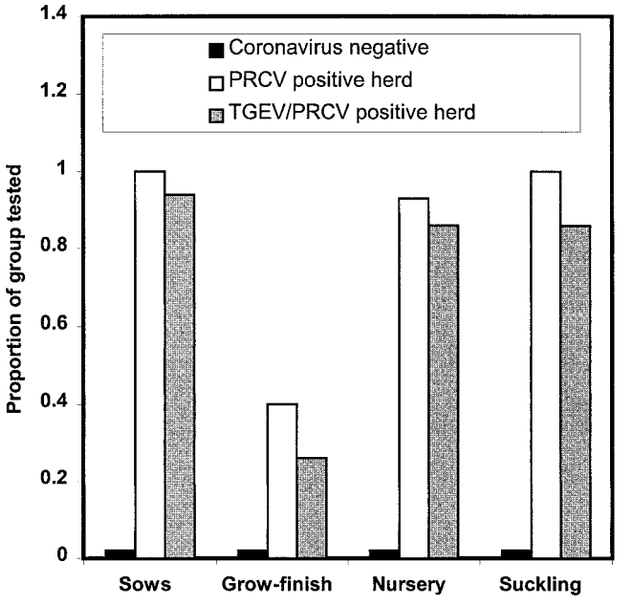

Figure 3. Proportion of pigs from different age groups and with different herd status with antibody to porcine coronavirus as determined by the transmissible gastroenteritis (TGEV)/porcine respiratory coronavirus (PRCV) ELISA.

in the evaluation. If grow-finish pigs were targeted for the monitoring program, where only 13% of the group may have TGEV antibody, a greater number of sera would need to be evaluated to detect positive animals. Conversely, to use the ELISA to diagnose active TGEV infection in an endemically infected herd, sera from pigs that had lost maternal antibody would need to be evaluated.

A single serum from pig 23, experimentally infected with PRCV, was initially incorrectly identified as positive for antibody to TGEV. However, in general, this commercial ELISA was repeatable, providing a high level of agreement between results when the same sera were tested on different days (kappa = 0.889) and when tested before and after heating (56 C for 30 minutes) (kappa = 0.888). Therefore, this ELISA can be used for the evaluation of sera that have been used for other serological assays, where heat inactivation may have already been performed.

The determination that 17/30 TGEV-infected commercial herds included pigs with antibody to TGEV and pigs with antibody to PRCV is similar to a previous report[18] and confirms that TGEV-infected herds can have individual animals with serum antibody to TGEV and other pigs with antibody to PRCV. In this study, sera sequentially collected from animals experimentally infected with TGEV were determined to be PRCV ELISA positive prior to 21 days postinfection. Similarly, sera from naturally TGEV-infected pigs with low VN antibody titers (<1:128) were more likely to be PRCV ELISA positive. This may help explain why both types of animals can be found in the same herd.

The ELISA had high sensitivity and specificity and good agreement with the VN assay for the identification of antibody to porcine coronaviruses (TGEV or PRCV), which suggests that the ELISA could be used alone for a herd monitoring program. Parallel use of the ELISA and VN did not substantially increase the sensitivity due to the significant sensitivity covariance. Therefore, when cost is an issue, the VN test can be eliminated. However, interpretation of results from individual herds may be made easier for veterinarians with parallel use of the VN assay, where antibody titers are reported. Alternatively, as demonstrated by the increasing percent inhibition for monoclonal antibodies following porcine coronavirus (TGEV and PRCV) infection of experimental pigs, the percent inhibition could be used as an estimate of the level of antibody in sera.

From an epidemiologic viewpoint, classification of a herd as infected or noninfected on the basis of test results depends on several criteria: prevalence of infected animals within the herd, herd size, number of animals tested, characteristics of individual tests, cutpoint used to declare a herd infected, and variation in individual-level test sensitivity and specificity.[1,10] Simulation models, based on probability distributions, have been developed to determine estimates of herd-level sensitivity and specificity of diagnostic tests.[1,9] Although not included in this article, application of a stochastic simulation model[9] provided a technique to review performance of this ELISA under a variety of assumptions. The ELISA test performed well in this simulation model.

This study suggests that this commercial blocking TGEV/PRCV ELISA can be applied to large populations of swine and would best be used on a herd basis on targeted age groups for the identification of TGEV-infected herds.

Sources and manufacturers

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