# Differentiation between transmissible gastroenteritis virus and porcine respiratory coronavirus using a cDNA probe

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**Abstract.** A plasmid, pG3BS, containing a cDNA clone from the 5' coding region of the peplomer glycoprotein gene appears to be specific for enteric transmissible gastroenteritis virus (TGEV) strains and for live-attenuated TGEV vaccines. This cDNA probe is used to differentiate porcine respiratory coronavirus (PRCV) isolates from TGEV field and vaccine strains by a slot blot hybridization assay. Probe pG3BS also hybridizes to canine coronavirus (CCV) RNA but does not hybridize to antigenically related feline infectious peritonitis virus (FIPV) RNA. The RNAs of 13 enteric TGEV isolates from the United States, Japan, and England, 4 US-licensed live-attenuated TGEV vaccines, and antigenically closely related CCV were detected by pG3BS. The RNAs of FIPV and 3 US isolates of PRCV did not react with pG3BS but were detected by a TGEV-derived plasmid, pRP3. Pigs infected with either PRCV or TGEV test serologically positive for TGEV antibody by the serum neutralization test. Characterization of the virus circulating in a swine herd by the pG3BS probe will differentiate between an enteric TGEV and a respiratory PRCV infection.

Transmissible gastroenteritis virus (TGEV) of swine is a coronavirus that was isolated for the first time in 1946.[4] The virus replicates in the cytoplasm of mature absorptive epithelial cells on the surface of villi in the small intestine. Transmissible gastroenteritis virus causes destruction of these epithelial cells, resulting in watery diarrhea and dehydration that leads to almost 100% mortality in susceptible pigs infected during the first few weeks of life. A TGEV variant, designated porcine respiratory coronavirus (**PRCV**), was first isolated in Belgium in 1984. In neonatal pigs, PRCV apparently causes a limited infection of subepithelial cells of the intestinal villi but does not spread to adjacent cells.[3] Porcine respiratory coronavirus does replicate extensively in epithelial cells of the upper and lower respiratory tract and in alveolar macrophages but only causes an inapparent infection.[12] Because PRCV-infected swine produce TGEV neutralizing antibodies, it is not possible to differentiate between a TGEV and a PRCV infection by the serum neutralization test. In Europe, a competitive inhibition enzyme-linked immunosorbent assay (ELISA) has been developed to distinguish swine herds with circulating PRCV or TGEV.[1,6] This blocking ELISA test is based on the specificity of monoclonal antibodies that recognize epitopes present on TGEV that are either modified or absent on PRCV. However, because the sensitivity of this test is low, some swine herds infected with TGEV may go undiagnosed.

Recently, we reported that a PRCV was present in the United States.[16] The virus was similar antigenically to the European PRCV, and both viruses cause only inapparent respiratory infections in neonatal swine. It is important for a swine producer with a herd serologically positive for TGEV antibodies to know if the herd was infected with PRCV or TGEV. In this paper, a cDNA clone derived from the 5' end of the TGEV peplomer glycoprotein gene is shown to hybridize with sequences conserved among TGEV vaccines and field strains but does not detect RNAs prepared from 3 US PRCV isolates.

## Materials and methods

*Viruses and cells.* The origins of the TGEV isolates, California, FS-216/64 (England), Hormel, Illinois, Iowa, Kansas, Miller, New York II, Purdue-37, and Shizuoka-25 (Japan), have been described? Additional TGEV field isolates from Nebraska[13] and Williamsburg (IA) were used. The Purdue-115 strain that grows to high titer was obtained,[a] and a small plaque variant of the Miller strain of TGEV that causes a persistent infection in a continuous leukocyte cell line was used.[17] The live-attenuated TGEV vaccine strains were commercially available products.[b,c,d,e] The UCD-1 strains of feline infectious peritonitis virus (FIPV) and canine coronavirus (CCV) were used.[19] The origin of the PRCV-Ind/89 isolate has been described.[16] Two additional field isolates of PRCV from North Carolina and Minnesota were isolated in 1989. All viruses were plaque purified except the 4 vaccine strains and the PRCV field isolates from North Carolina and Minnesota.

The TGEV and PRCV isolates were grown on a swine testicular (ST) cell line, and CCV and FIPV were grown on fetal cat cells (FC) as described.[18,19]
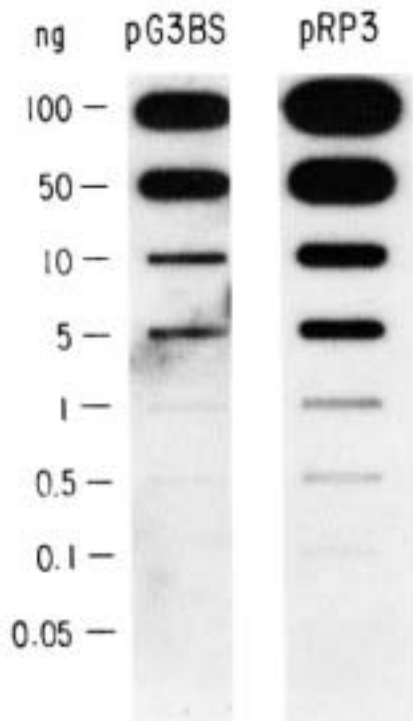
**Figure 1.** Slot blot hybridization of virus RNA from sucrose gradient purified TGEV virions of the Purdue-115 strain. Nick-translated cDNA probes, pG3BS and pRP3, were used to detect different concentrations of the viral RNA. Blots were autoradiographed for 3 days at -70 C.

*Virus purification.* Confluent ST cells in 12 flasks (75 cm$^2$) were infected with the Purdue-115 strain of TGEV at a multiplicity of infection of approximately 10 plaque forming units/cell. The virus from clarified culture supernatants was concentrated by centrifugation (SW28 rotor)[f] at 113,000 x g at 4 C for 18 hr through a discontinuous sucrose gradient consisting of 12 ml 30% (w/w) and 8 ml 50% (w/w) sucrose solutions prepared in TEN-Tween 20 (1 M NaCl, 1 mM ethylene diaminetetraacetic acid [EDTA], 0.01 M Tris HC1 pH 7.4, 0.02% Tween 20 [v/v]). Virus from the 30/50% sucrose interface was collected, diluted 1:3 in TEN-Tween 20, and pelleted in the SW 41 rotor[f] 154,000 x g at 20 C for 3 hr. Each virus pellet was resuspended in 200 µl of 0.14 M NaCl, 1.5 mM MgCl$_2$, 10 mM Tris HC1 pH 8.6, 0.5% Nonidet P-40, 1 mM dithiothreitol, and 100 units/ml placental RNase inhibitor[g] prior to extraction of the genomic RNA.

*RNA isolation.* Viral genomic RNA was prepared from the resuspended virus pellets by adding an equal volume of 0.3 M NaCl, 0.2 M Tris HC1 pH 8.0, 25 mM EDTA pH 8.0, and 2% sodium dodecyl sulfate (SDS). Proteinase K was added to a final concentration of 100 µg/ml, mixed, and incubated for 30 min at 37 C. Disrupted virions were extracted 1 time with an equal volume of phenol: chloroform and ethanol precipitated at -20 C overnight. The RNA was resuspended in distilled water, and the concentration was determined spectrophotometrically where 1 OD$^{260}$ = 40 µg/ml.[9]

Total intracellular RNAs were prepared from coronavirus-infected ST cells, FC cells, or from persistently infected swine leukocytes by guanidinium isothiocyanate extraction and CsCl cushion centrifugation. [15]

*Plasmids.* Plasmid pRP3 (3232 base pairs [bp]) is a cDNA clone of the Miller PP3 isolate of TGEV cloned into the EcoR1 site of the pBluescript phagemid.[h] Plasmid pG3BS (396 bp) is a subclone of Miller TGEV cDNA clone pRP1[14] that contains sequences from the 5' coding region of the peplomer gene.

*Slot blot hybridization.* For slot blot hybridization, 0.1-3.2 µg/well of coronavirus-infected cellular RNA was applied to a nylon membrane[i] in a slot-blot apparatus[j] previously treated with 0.1% diethylpyrocarbonate.[9] The dried nylon membrane was placed on filter paper saturated with 0.025 M sodium phosphate pH 6.0 for 30 set and UV cross-linked to covalently bind the RNA to the nylon membrane.[2] Prehybridization was carried out at 65 C for 3 hr in 6 x standard saline citrate (SSC, which is 0.15 M NaCl, 0.015 M Na citrate, pH 7.0), 5 x Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin), 0.5% SDS, and 100 µg/ml of sonicated denatured salmon sperm DNA.[9] Hybridization was carried out at 65 C for 18 hr in fresh prehybridization solution containing 5 x 10$^6$ counts per minute of nick-translated $^{32}$P-labeled plasmid pG3BS. After incubation, membranes were washed 3 times for 5 min each in 2 x SSC and 0.1% SDS at room temperature, followed by 2 high-stringency washes in 1 x SSC and 0.1% SDS at 68 C for 1 hr. Membranes were kept moist and exposed to X-OMAT film[k] at - 70 C with an intensifying screen[l] for 1-7 days.

After autoradiography, membranes were stripped of radioactivity to be reused with the second probe, pRP3. Stripping was accomplished by boiling for 30 min in 0.1 x SSC and 3% SDS, followed by a 6 x SSC rinse. At this point, membranes were checked by autoradiography (overnight exposure) for residual radioactivity prior to proceeding with another round of prehybridization and hybridizations using a second $^{32}$P-labeled probe.

## Results

Two cDNA clones prepared from genomic RNA of the virulent Miller strain of TGEV were used as probes. One cDNA probe, pG3BS (396 bp), was derived from the 5' end of the peplomer glycoprotein gene. The other probe, pRP3 (3232 bp), contained sequences from the 3' end of the peplomer gene, the genetic region between the peplomer and integral membrane gene, and the entire integral membrane gene sequence except for the last 12 bases at the 3' end. Whole plasmids containing both insert and vector were labeled with $^{32}$P and used as probes.

Genomic TGEV RNA was used to determine the sensitivity of the slot blot hybridization assay. Probe pG3BS detected TGEV RNA sequences at the 0.5 ng level, whereas the larger pRP3 probe was approximately 5 times more sensitive detecting TGEV RNA at the 0.1 ng level (Fig. 1).

The intracellular RNAs prepared from coronavirus-infected cells were cross-linked onto nylon membranes at 2 concentrations. The concentrations of intracellular
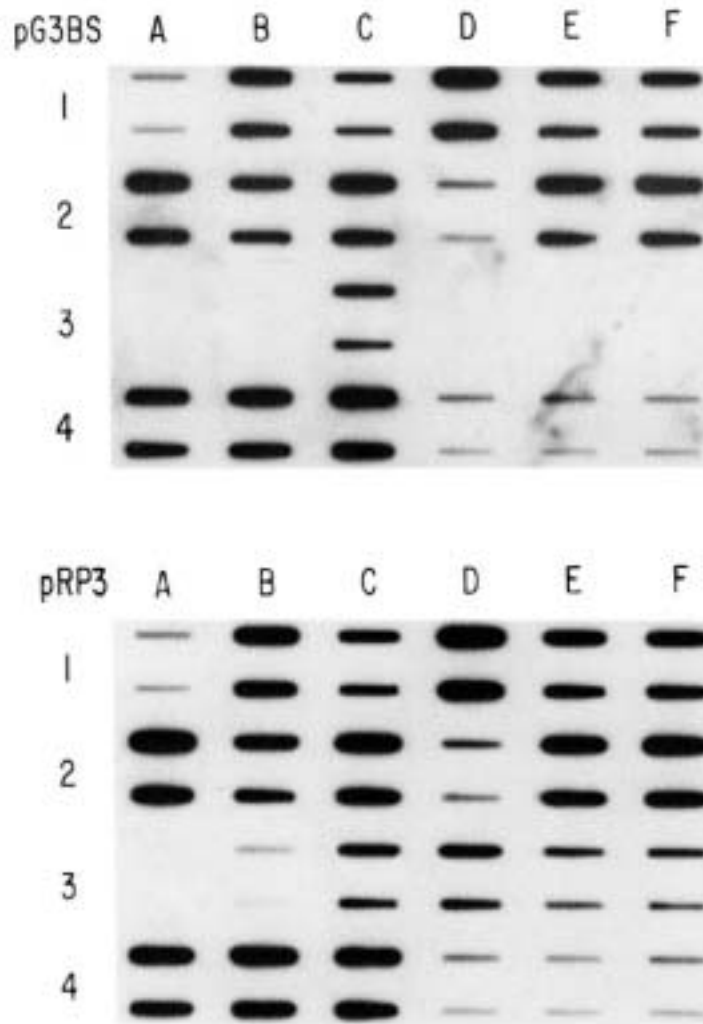
**Figure 2.** Slot blot hybridizations of total RNA from coronavirus-infected cells. Each RNA preparation was applied to the membrane at 2 concentrations-the upper application had twice the amount of RNA as the lower application. Coronavirus-infected cellular RNAs are 1A, small plaque TGEV variant; 2A, Miller isolate; 3A, control ST cell RNA; 4A, vaccine;[b] 1B, California isolate; 2B, high passage Miller-60; 3B, FIPV RNA; 4B, vaccine;[c] 1C, England isolate FS-216/64; 2C, New York-II isolate; 3C, CCV RNA; 4C, vaccine;[d] 1D, Hormel isolate; 2D, Purdue-37; 3D, PRCV-Ind89; 4D, vaccine;[e] 1E, Illinois isolate; 2E, Shizuoka isolate; 3E, PRCV-Minn/89; 4E, Williamsburg isolate; 1F, Iowa isolate; 2F, Nebraska isolate; 3F, PRCV-N.C./89; and 4F, Kansas isolate. The membranes were hybridized first with pG3BS, then stripped of radioactivity and reprobed with pRP3.

RNA applied to the membranes ranged from 0.1 to 3.2 μg/well because the amount of viral specific RNA in each preparation was different. The RNAs were prepared from 13 different enteric TGEV isolates, 3 PRCV isolates, 4 licensed live-attenuated TGEV vaccines, FIPV, and CCV. Uninfected ST cell RNA was used as a control. The blotted membrane was first reacted with the pG3BS probe, stripped, and then hybridized to labeled pRP3 (Fig. 2). The pG3BS probe detected all coronavirus RNAs except the PRCV and FIPV RNAs. Reprobing the same membrane with pRP3 (Fig. 2) showed that adequate amounts of PRCV RNA and FIPV RNA were applied to the membranes but were not detected with the pG3BS probe. Neither probe reacted with the ST cell control RNA.

## Discussion

In this study, a cDNA clone, pG3BS, identified at the 5' end of the peplomer glycoprotein gene appears to be specific for enteric TGEV strains and for live-attenuated TGEV vaccines. This probe is used to differentiate PRCV isolates from TGEV field and vaccine strains by a slot blot hybridization assay. Also, pG3BS hybridizes to CCV RNA and does not hybridize to FIPV RNA.

To determine if a swine herd is infected with either PRCV or TGEV using probe pG3BS, the virus must first be isolated and the viral RNA analyzed by the hybridization assay. However, if some animals are known to be shedding virus, then hybridization analysis of nasal swab material should be sufficient.[10] Cur-

rently in the United States, no serologic tests are available to distinguish between PRCV- and TGEV-infected swine herds. A herd testing positive for TGEV antibody could have been infected with either virus.

Because of the discontinuous nature of RNA replication in coronaviruses, RNA-RNA viral recombinants and deletion mutants are produced. For example, RNA-RNA recombination within the peplomer gene probably accounts for some of the biological differences between FIPV and TGEV. The nucleotide sequence homology between TGEV and FIPV was only 39% at the 5' end of the peplomer gene up to amino acid residue 274.[7] After residue 274, the peplomer gene sequence homology was 93%, suggesting that a recombination event occurred in the vicinity of amino acid residue 274. Because probe pG3BS is derived from the peplomer gene region with low homology, there is a lack of hybridization of probe **pG3BS** to FIPV RNA. In the case of another coronavirus, mouse hepatitis virus (MHV), deletion mutants have occurred spontaneously or after selection with neutralizing monoclonal antibodies[5] These large deletions, 426-477 nucleotides, were localized at the 5' coding region of the MHV peplomer gene.[11] The deletions appeared to enhance the ability of MHV to replicate in cell culture. Similar deletions downstream from the TGEV peplomer gene have generated viral variants with altered phenotypes.[15] Under conditions of high stringency, probe **pG3BS** hybridized to the RNAs of 13 different TGEV isolates and 4 vaccine strains. This indicates that nucleotide sequences at the 5' end of the TGEV peplomer gene are conserved. The lack of hybridization of probe **pG3BS** to the 3 US isolates of PRCV could be due to either nucleotide sequence divergency as seen for FIPV or to large deletions similar to those that occur in MHV.

## Sources and manufacturers

a. Provided by Dr. Linda Saif, Ohio Agricultural Research and Development Center, Wooster, OH.
b. Fort Dodge Laboratories, Inc., Ft. Dodge, IA.
c. Diamond Laboratories, Inc., Des Moines, IA.
d. Ambico, Inc., PO Box 522, Dallas Center, IA.
e. Solvay Animal Health, Inc., 2000 Rockford Rd., Charles City, IA.
f. Beckman Instruments, Inc., PO Box 10200, Palo Alto, CA.
g. Promega Corp., 2800 S. Fish Hatchery Rd., Madison, WI.
h. Stratagene Cloning Systems, 11099 N. Torrey Pines Rd., La Jolla, CA.
i. Gene Screen Hybridization Transfer Membrane, NEN Research Products, 549 Albany St., Boston, MA.
j. Bio-Rad Laboratories, 2200 Wright Ave., Richmond, CA.
k. Eastman Kodak Co., Rochester, NY.
l. E. I. DuPont de Nemours & Co., Wilmington, DE.

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