Effect of coinfection with genogroup 1 porcine torque teno virus on porcine circovirus type 2–associated postweaning multisystemic wasting syndrome in gnotobiotic pigs

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Objective—To determine whether genogroup 1 porcine torque teno virus (g1-TTV) can potentiate clinical disease associated with porcine circovirus type 2 (PCV2).

Sample population—33 gnotobiotic baby pigs.

Procedures—Pigs were allocated into 7 groups: group A, 5 uninoculated control pigs from 3 litters; group B, 4 pigs oronasally inoculated with PCV2 alone; group C, 4 pigs inoculated IP with first-passage g1-TTV alone; group D, 4 pigs inoculated IP with fourth-passage g1-TTV alone; group E, 6 pigs inoculated IP with first-passage g1-TTV and then oronasally inoculated with PCV2 7 days later; group F, 6 pigs inoculated IP with fourth-passage g1-TTV and then inoculated oronasally with PCV2 7 days later; and group G, 4 pigs inoculated oronasally with PCV2 and then inoculated IP with fourth-passage g1-TTV 7 days later.

Results—6 of 12 pigs inoculated with g1-TTV prior to PCV2 developed acute onset of postweaning multisystemic wasting syndrome (PMWS). None of the pigs inoculated with g1-TTV alone or PCV2 alone or that were challenge exposed to g1-TTV after establishment of infection with PCV2 developed clinical illness. Uninoculated control pigs remained healthy.

Conclusions and Clinical Relevance—These data implicated g1-TTV as another viral infection that facilitates PCV2-induced PMWS. This raises the possibility that torque teno viruses in swine may contribute to disease expression currently associated with only a single infectious agent. (*Am J Vet Res* 2008;69:1608–1614)

Infection with PCV2 and its primary disease manifestation, PMWS, is recognized as an important disease problem of swine throughout the world.[1–3] An epidemic form of PMWS was identified in eastern Canada,[4,5] and the incidence of the disease complex is increasing sharply in the United States.[5] Postweaning multisystemic wasting syndrome is characterized by a constellation of clinical signs that includes progressive weight loss, jaundice, and pneumonia.[1–3] Losses in affected herds can be substantial, with deaths of up to 40% of affected weanling pigs. Gross lesions consist of generalized lymphadenopathy, hepatitis, nephritis, and pneumonia. Histologically, the disease is characterized by widespread granulomatous inflammation, formation of histiocytic syncytial cells, and variable numbers of intracytoplasmic basophilic viral inclusions within infiltrating histiocytes and macrophages.[1,3,6,7] Although the primary cellular tropism of PCV2 has yet to be precisely defined, particularly during the earliest phases of infection, it is clear that many epithelial and mesenchymal cell types, including those of hematopoietic lineage cells, can support productive infection and that infectious virus progressively accumulates within viable macrophages in lymph nodes and elsewhere.[1,2]

Although PCV2 is currently considered necessary to cause PMWS and the clinical disease syndromes associated with PCV2,[1,3,8,9] PCV2 in a herd (as determined by use of results of serologic testing, PCR assay, or vi-

Abbreviations

g1-TTV	Genogroup 1 torque teno virus
g2-TTV	Genogroup 2 torque teno virus
nPCR	Nested PCR
PCV2	Porcine circovirus type 2
PCVD	Porcine circovirus–associated disease
PPV	Porcine parvovirus
PRRSV	Porcine reproductive and respiratory syndrome virus
PMWS	Postweaning multisystemic wasting syndrome
TTV	Torque teno virus

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rus isolation) is frequently not sufficient to manifest as PCVDs. Numerous cofactors have been identified as having a role or roles in the genesis of PCVDs. Cofactors appear to facilitate viral replication of PCV2 and enhance clinical expression of PCVDs when they are introduced into PCV2-infected swine.[1,2,8–11] The first infectious agent identified as a cofactor for PMWS was PPV, a DNA virus of minimal pathogenicity in young swine.[9,10] Infection with PRRSV is a clearly established cofactor for disease expression by PCV2.[1,2,10–13] Colonization of the respiratory tract by *Mycoplasma hyopneumoniae* preferentially promotes PCV2-associated granulomatous pneumonia.[14] Other infectious cofactors have been identified and reviewed elsewhere.[1,3,10,12,13]

The most probable common feature among all of the infectious cofactors is dysregulation of immune responses during the peak replication phase of infection or during convalescence as the infection abates. Immunosuppression or immunologic activation, the latter of which is also known as reactive systemic lymphoid hyperplasia, appears to facilitate PCV2 replication in lymphoid tissues. Injections with immunogens emulsified in a potent adjuvant (incomplete Freund's adjuvant) mimics this lymphoproliferative reaction and has been used to induce PMWS in PCV2-infected gnotobiotic baby pigs in the absence of other infectious disease agents.[15] This effect is also seen in pigs vaccinated with certain *M hyopneumoniae* bacterins in both experimental[16] and field[17] conditions.

Many outbreaks of PMWS have not been associated with known infectious disease agents or changes in management or vaccinations, an observation that suggests additional unidentified cofactors are operable in the disease complex. A positive association between viremia attributable to g2-TTV and PMWS in a cohort of European swine has been reported.[18] The TTVs are ubiquitous viral DNA agents in the same viral family (Circoviridae) as PCV2.[18–36] The TTVs recovered from humans,[22,24] nonhuman primates,[25,26] and cats, cattle, and swine[26–28] are tentatively assigned to the genus *Anellovirus* within the Circoviridae family. Similar to PCV2, the genome of TTVs is a single-stranded circularized DNA molecule.[28] However, in contrast to PCV2, but similar to chick anemia virus, the untranslated region containing the transcription initiation sequence does not contain the stem loop start sequence characteristic of the porcine circoviruses.[19,20,22,23,28] Swine TTVs, similar to human TTVs, have not been cultured in vitro, and the only method of identification of infected patients is by use of nPCR or direct PCR assays.[18,26–31] As a group, the TTVs are genomically diverse.[19–21,23,32–34] On the basis of genotypic homology, at least 5 human genogroups are known; the swine TTVs have been divided into 2 genogroups (ie, g1- and g2-TTV).[28] There is a high incidence of infection (viremia as determined by use of PCR assays) in clinically normal swine (and humans), and all attempts to associate TTV infection with disease processes have been frustrated by the high prevalence of TTV viremia in control populations. The general consensus is that TTVs are nonpathogenic orphan viruses of minimal or no pathogenic potential that are a part of the normal biota of their host species.[19–21,23,35,36]

In another study[37] conducted by our laboratory group, we reported that porcine g1-TTV can be transmitted to gnotobiotic swine via TTV-positive plasma obtained from conventional pigs and that gnotobiotic pigs infected with in vivo–passaged TTV develop mild interstitial pneumonia. The purpose of the experiments reported here was to determine the potentiating effects of g1-TTV on PCV2-induced PMWS in gnotobiotic swine. Furthermore, we assessed the sequence of infections in dual-infected gnotobiotic pigs as a critical determinant of clinically expressed disease.

Materials and Methods

Animals—A total of 33 gnotobiotic[38] pigs were derived by cesarean section from 5 sows. Baby pigs were placed into sterile pen-tub isolation units (3 to 6 pigs/U). Each group of pigs was housed in separate self-contained isolation units and fed a commercial sow milk replacer, as described elsewhere.[38] Prior to and at the conclusion of the experiments, samples from each isolation unit (food, feces, and cages) were cultured for aerobic and anaerobic bacterial growth to confirm gnotobiotic status; all cultures were negative for bacterial colonies. Animal experiments were approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

Viruses—The fourth in vivo passage of the Stoon 1010 isolate of PCV2 (ie, OSU/PCV2p4) was used as the source of PCV2 challenge inoculum. This 10% (wt/vol) tissue pool contained 5×10^8 PCV2 infectious U/mL and had negative results for g1- and g2-TTV DNAs when tested by use of an nPCR assay.[18] As described elsewhere,[37] porcine g1-TTV was recovered from young conventional swine by IP injection of g1-TTV DNA-positive pooled plasma into gnotobiotic baby pigs. The liver and bone marrow homogenates positive for g1-TTV DNA were extracted with chloroform[39] to eliminate all enveloped viral pathogens and then serially passaged through 2 additional generations of gnotobiotic pigs; chloroform extraction was used on passages 1 through 3 (ie, g1-cTTVp1 through g1-cTTVp3, respectively). A 10% (wt/vol) homogenate of passage 4 homogenate (ie, g1-TTVp4) was not extracted with chloroform; however, the homogenate was divided into 2.0-mL aliquots and frozen at –70°C for subsequent in vivo challenge experiments.

Experimental design—The 33 gnotobiotic pigs were allocated into 7 groups: group A, 5 uninoculated control pigs from 3 litters; group B, 4 pigs oronasally inoculated with PCV2/OSUp4 alone; group C, 4 pigs inoculated IP with g1-TTVp1 alone; group D, 4 pigs inoculated IP with g1-TTVp4 alone; group E, 6 pigs inoculated IP with g1-TTVp1 and then oronasally inoculated with PCV2/OSUp4 7 days later; group F, 6 pigs inoculated IP with g1-TTVp4 and then inoculated oronasally with PCV2/OSUp4 7 days later; and group G, four 3-day-old pigs inoculated oronasally with PCV2/OSUp4 and then inoculated IP with g1-TTVp4 7 days later. Day of initial inoculation was designated as day 0. Blood samples were collected before inocula-

tion, at weekly intervals, and at the time of euthanasia, and serum was harvested. Pigs were observed for signs of illness at least 3 times/d. Pigs were euthanized by IV administration of an overdose of barbiturate when moribund or on days 32 to 34 after inoculation.

PCR assays—The DNA was extracted from frozen (–70°C) sera after thawing; extraction was performed by use of conventional procedures. For routine screening of samples for g1-TTV DNAs, an nPCR assay with published[18,28] primer sequences for g1-TTV was used, as described elsewhere.[37] Assessment of viremia attributable to infection with PCV2 was accomplished by PCR assay.[40,41]

Histologic examination and immunohistochemical analysis—Samples of the lungs, liver, kidneys, ileum, spleen, thymus, and superficial inguinal, axillary, bronchial, and mesenteric lymph nodes were collected into cold (4°C) 100% ethanol. Tissue samples were fixed for 24 hours at 4°C and then processed for histologic examination by use of routine methods. Rehydrated section replicates were stained with H&E to detect PCV2 nucleocapsid protein.[7–9,15] Section replicates of the kidneys were also stained with monoclonal antibodies[a,b] and developing reagents in kit form[c] to detect deposits of porcine fibrinogen or fibrin and porcine IgG. Briefly, 5-µm-thick deparaffinized sections were quenched for endogenous peroxidase with 3% (vol/vol) hydrogen peroxide in PBS solution, reacted with the appropriate monoclonal antibody, blocked with 4% (vol/vol) heat-inactivated horse serum, reacted with biotinylated horse anti-mouse IgG, and stained with avidin-peroxidase and 3,3′diaminobenzidine.

Results

Group A—All 5 uninoculated control pigs were clinically normal at the end of the experiment, and histologic changes in tissues were compatible with those listed in other reports.[7–9,15] Tissue section replicates had negative results for PCV2 nucleocapsid protein, and sera obtained before inoculation and immediately before euthanasia had negative results for both PCV2 and g1-TTV DNAs by use of immunohistochemical analysis and nPCR assay, respectively.

Group B—All 4 pigs in this group remained healthy but were PCV2 DNA viremic as determined by use of PCR assay at the conclusion of the experiment. In this group, modest bronchial lymphadenopathy was detected in 3 of 4 pigs, and all 4 pigs had mild generalized lymphadenopathy and mild thymic atrophy. Histologically, lymphoid hyperplasia (germinal centers, hyperplasia of T-cell–dependent areas, and modest reticuloendothelial cell hyperplasia) accounted for the lymphadenopathy. A few syncytial giant cells were evident in a lymph node of 1 pig; all pigs had modest mononuclear inflammatory cell infiltrates in the liver. Tissue sections stained for PCV2 nucleocapsid protein revealed scattered small foci or single mononuclear cells that contained reaction product. These foci were restricted to lymphoid tissues or a few inflammatory cell infiltrates in the liver and lungs, but were most apparent in bronchial lymph nodes.

Groups C and D—The 4 pigs inoculated with g1-TTVp1 alone remained healthy and were euthanized on day 32. Histologic examination revealed that lymphoid tissues were hypoplastic, with the only histologic changes being rare formation of germinal centers in bronchial and mesenteric lymph nodes, which was similar to the results for uninoculated control pigs of group A. Three of 4 pigs had a few inflammatory cell infiltrates in the liver parenchyma and diffuse interstitial pneumonia. In the kidneys, renal glomeruli were modestly distended with eosinophilic extracellular protein (or proteins) and segmental glomerular sclerosis. Three of 4 pigs had strong positive results for g1-TTV DNA by use of an nPCR assay in serum samples obtained on day 20; 1 pig had a trace positive result for g1-TTV DNA. Serum samples obtained immediately before euthanasia from all 4 pigs inoculated with g1-TTVp1 had negative results for PCV2 DNA.

All 4 pigs of group D were clinically normal at the conclusion of the experiment. Results similar to those for pigs of group C were evident in the group D pigs, except that the renal glomerular lesions were slightly more severe and the interstitial pneumonia was slightly more pronounced; all 4 pigs of group D had strong positive results for viremia against g1-TTV DNA and negative results for viremia against PCV2 by use of PCR assays in serum samples obtained immediately before euthanasia.

Group E—Three of 6 dual-inoculated pigs developed acute fatal PMWS. One pig died suddenly 18 days after inoculation with PCV2; 2 more pigs became moribund and were euthanized 19 days after inoculation with PCV2. All 3 PMWS-affected pigs had generalized lymphadenopathy, thymic atrophy, ascites, and small pale to yellow livers. Profound lymphoid depletion with replacement by histiocytes and sheets of granulomatous inflammation completely replaced all remnants of B- and T-cell zones in lymphoid tissues. Massive and diffuse hepatocyte necrosis was evident in all 3 pigs.

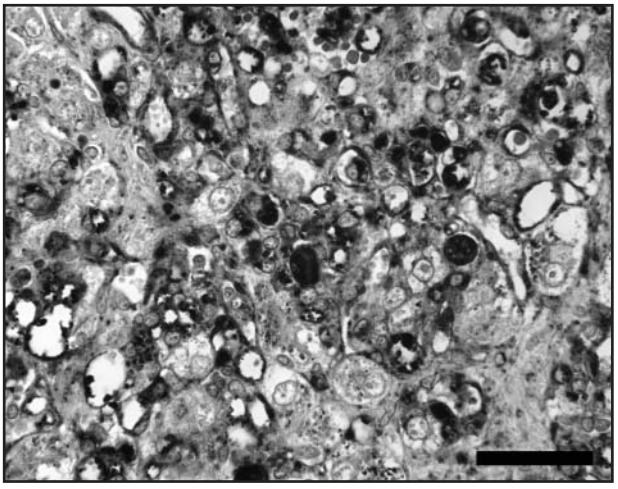

Figure 1—Photomicrograph of a section of liver obtained from a gnotobiotic pig inoculated with g1-TTV followed 7 days later by inoculation with PCV2 and subsequently developed PMWS (19 days after PCV2 inoculation). Viral (PCV2) nucleocapsid protein is evident in the cytoplasm and cell nuclei of hepatocytes and infiltrating macrophages. Immunohistochemical stain for PCV2 nucleocapsid protein; bar = 0.32 µm.

Section replicates stained for PCV2 nucleocapsid protein revealed overwhelming and diffuse infection of hepatocytes (**Figure 1**). Other epithelia (eg, bronchial and tonsillar) and enterocytes overlying Peyer's patches in the ileum contained PCV2 nucleocapsid protein. In kidney sections, tubular epithelial cells had positive results for virus. In addition, the renal glomeruli had pronounced linear deposits of PCV2 nucleocapsid protein, which was consistent with the morphology of the immune complexes (**Figure 2**). These glomerular deposits also had positive results for IgG and fibrin or fibrinogen (data not shown).

The 3 remaining pigs in this group survived until the conclusion of the experiment on day 32. In these pigs, gross lesions typical of moderate to severe subclinical PCV2 infection were detected. Generalized lymphadenopathy was evident, but the thymus of each of the 3 pigs was within the anticipated gross size limit. One pig had mild ascites, and another pig had a slightly pale liver. In all subclinically infected pigs, the lungs remained inflated and firm, which was suggestive of interstitial pneumonia. Healed small renal cortical infarcts were evident in 2 pigs. Histologic examination and immunohistochemical analysis revealed that all 3 pigs were actively infected with PCV2. Sera obtained at the time of euthanasia from all 6 pigs of group E contained both g1-TTV and PCV2 DNAs.

Group F—Six pigs comprised this group. On day 18 after PCV2 inoculation, 1 pig had a sudden onset of anorexia; this pig was euthanized on day 19 with clinical evidence of PMWS (icterus, edema, and ascites). A second pig died suddenly on day 25. In that pig, extensive pleural effusion and pulmonary edema associated with severe diffuse interstitial pneumonia were the apparent causes of death. A third pig developed subcutaneous edema on day 21; the pig became anorectic and was euthanized on day 32. All 3 affected pigs of group F had generalized lymphadenopathy, thymic atrophy, and small pale to yellow livers, which are typical of PMWS in gnotobiotic swine. Three of the 6 pigs in this group survived until completion of the experiment and were euthanized on day 34. One of these pigs had mild subcutaneous edema and ascites; the remaining 2 dual-inoculated pigs were healthy but had gross and histologic lesions compatible with subclinical PCV2 infection. Results of histologic examination and immunohistochemical analysis of the tissues from the pigs of group F were similar to those for pigs in group E, except that the pig with pleural effusion had extensive cellular infiltrates, which had positive results for PCV2 nucleocapsid protein in pulmonary interstitial and interlobular connective tissue septae.

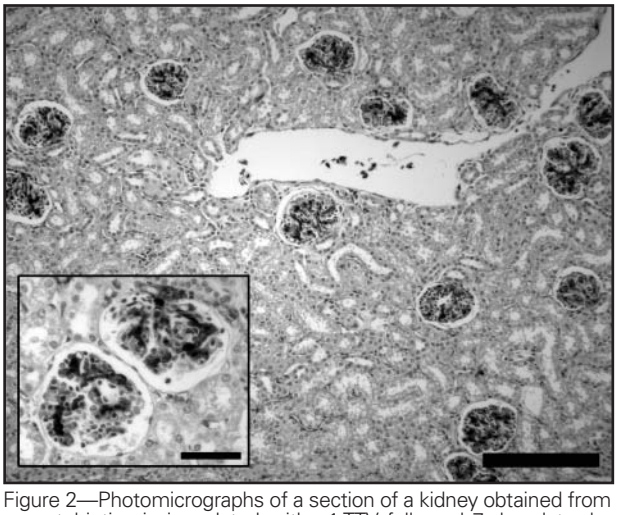

Figure 2—Photomicrographs of a section of a kidney obtained from a gnotobiotic pig inoculated with g1-TTV followed 7 days later by inoculation with PCV2 and subsequently developed PMWS (19 days after PCV2 inoculation). Tissues were immunohistochemically stained for PCV2 nucleocapsid protein. The renal glomerulus contains PCV2 nucleocapsid protein entrapped in the glomerulus and glomerular basement membrane in a pattern reminiscent of immune complex glomerulonephritis. Bar = 0.65 µm. Inset: higher-power magnification of the same section illustrating the discontinuous nature of the glomerular deposits, which are reminiscent of immune complex glomerulonephritis. Bar = 0.32 µm.

Group G—All 4 pigs survived dual inoculation and were clinically normal at the end of the experiment. Gross and histologic lesions in these pigs were compatible with stable subclinical PCV2 infection in that the thymus of each pig was of typical size and cellularity and lymphadenopathy was attributable to development of the germinal centers and lymphoreticular hyperplasia. Immunohistochemical analysis revealed that the PCV2 nucleocapsid protein antigen was restricted to single cells in lymphoid tissues with histiocytic or large lymphocyte morphologies. All 4 pigs were viremic for both PCV2 and g1-TTV at the conclusion of the experiment, as determined by use of results of PCR assays.

Discussion

Postweaning multisystemic wasting syndrome is recognized as an important and emerging devastating viral infectious disease of pigs that is currently in all major swine-producing areas of the world. The primary organ system affected (pulmonary, enteric, or renal) and the mortality rate vary among pigs and herds of pigs, but a predominant clinical finding in affected swine is a wasting syndrome characterized by generalized lymphadenopathy, persistent fever, anorexia, progressive weight loss, and an increase in the incidence of deaths attributable to other infectious bacterial and viral agents of swine. Frequently, pigs identified as survivors of PMWS also fail to thrive and have poor weight gains, and as a result, they represent additional economic losses to producers. Both virologic and pathologic evidence have identified PCV2 as the causal virus of PMWS.[1,3] Moreover, reports[42,43] highlight the dramatic efficacy of inactivated PCV2 vaccines in preventing PMWS in problem herds. This important observation further solidifies the central role that PCV2 plays in the expression of PMWS and the PCVDs.

For many swine veterinarians and veterinary pathologists, it has been difficult to fully accept PCV2 as a pathogen in pigs, partly because of the high incidence of subclinical PCV2 infection in clinically normal pigs[1,3,40,41] and partly because the critical early events in the pathogenesis of PMWS are still not completely understood. Difficulties in reproducing PCVD in pigs by use of PCV2 alone have also contributed to this skepticism. Reliable reproduction of PMWS in PCV2-infected swine appears to require a cofactor or cofac-

tors for full disease expression. The first indication of this was the establishment of the role that PPV plays in facilitating PMWS.[8,9] Indeed, PPV is used by other investigators as the method of choice for induction of PCV2-associated PMWS.[1] Other infectious agents of swine, notably PRRSV,[1,3,11,44] *M hyopneumoniae*,[14] and perhaps other agents,[10,12,13] have been incriminated as cofactors for expression of PMWS. All of these coinfections exert immunologic dysregulation, in most cases as immunostimulatory effects on the immune system. By use of this concept as a guide, the cofactor effect has been reproduced in pigs inoculated with PCV2 alone by immunization with antigen emulsified in mineral oil adjuvant[15] and by vaccination with mineral oil–containing bacterins, with the latter in experimental[16] and field conditions.[17]

In the experiments reported here, infection with porcine g1-TTV appears to facilitate development of PMWS in PCV2-infected gnotobiotic pigs. Although these data indicate that the TTVs should be added to the group of infectious agents that promote PMWS, the mechanism or mechanisms by which g1-TTVs promote disease are obscure and appear to differ from those in other coinfection methods of PMWS in which immunostimulation is regarded as central to promotion of PMWS. In contrast, pigs infected with g1-TTV have minimal or no histologic evidence for immunologic activation that could be attributable to viral infection. Moreover, the order of the coinfection with g1-TTV and PCV2 appears to determine clinical outcome. When TTV infection was initiated after PCV2 infection was established, the TTVs did not promote PMWS (0/4 pigs were affected), whereas g1-TTV infection prior to infection with PCV2 did promote PMWS (6/12 pigs were affected).

Other differences also exist. Whereas the latency period varies within the PPV or immunization-induced methods for induction of PMWS, clinical expression is evident between 4 and 5 weeks after inoculation with PCV2.[9,15] In the case of TTV, PMWS was expressed as early as day 18 after PCV2 inoculation (mean, day 23 after inoculation) or approximately 2 weeks earlier than was expected. In addition, there was rapid expansion of PCV2 infection from lymphoid tissues to epithelia (enteric, respiratory, renal tubular, and hepatic epithelial cell types). In this regard, the TTV method is more similar to the cyclosporine-mediated immunosuppression method for induction of PMWS.[7] Currently, it is difficult to assign the apparent potentiating effects of TTV to the immunologic dysregulation method for induction of PMWS, and the pathway or pathways whereby TTV promotes PCV2 infection remain undefined. Regardless of the mechanism, the potentiating effects mediated by g1-TTV were unexpected and certainly could not have been predicted given the lack of lymphoreticular hyperplasia associated with g1-TTV infection alone.

We used 2 in vivo passages of g1-TTV in the PCV2-PMWS potentiation experiments reported here. The only difference between the first and fourth passage was the amount of g1-TTV DNAs contained within each. Quantitative PCR assays revealed that in vivo passage 4 contained approximately 10^4 g1-TTV DNA copies/ng of total DNA, whereas passage 1 contained 10^1 to 10^2 g1-TTV DNA copies/ng of total DNA.[37] We anticipated that the PCVD-facilitating effects of g1-TTVp4 might be greater than those for g1-TTVp1, but it was not. Perhaps if these experiments had been expanded to include more pigs in each challenge group (eg, 10 to 15 pigs/group), a significant difference in the effects between these 2 passages of g1-TTV would have been detected.

Little is known about the biological aspects of the porcine TTVs. In 1 report[18] from Europe, investigators detected a high incidence of viremia associated with g2-TTV in pigs affected with PMWS, although cause-and-effect relationships could not be established because these pigs were field cases of PMWS. We know that porcine g1- and g2-TTV may be acquired via in utero infection and presume that both can cause infections by other routes as well. Currently, the potential virulence of g2-TTV is not known. It may behave similarly to g1-TTV (as indicated here), or it may be more or less virulent than g1-TTV. Comparative challenge studies will be needed to determine the relative virulence of these 2 viruses and, hence, their relative importance in infectious diseases of swine.

Given the apparent ease of parenteral transmission of the TTVs, the fact that at least 2 porcine TTV genogroups[18,28] exist as endemic infections in swine,[18,29–31] and the possibility that the genogroups may differ in pathogenicity for swine,[18] it is possible that the TTVs are an unknown potentiating factor, designated by some as agent X. Although epidemiologic studies in Sweden[45] and Denmark[46] have not implicated infectious agents other than PCV2 in the genesis of PMWS, the best evidence for the existence of such an infectious agent X has been found in an epidemiologic study[47] performed in the United Kingdom. Starting in 2000, these data trace the spread of epidemic PMWS from farms in Cornwall in the southwest of England to the north and east during several years. Risk factors for contracting PMWS were identified as size (farms with > 600 sows) and movement of pigs and farm visitors.

From the epidemiologic data from the United Kingdom, certain characteristics of an agent X can be inferred. Analysis of data suggests that agent X was transmitted among farms by contact and that the properties of agent X include resistance to environmental conditions and common disinfection procedures. Furthermore, agent X is likely to cause a swine-associated infection without a clinical or pathologic footprint that is sufficiently distinct from the clinical signs or lesions of other known pathogens of pigs. Thus, the presence of agent X would escape detection by conventional pathologic, serologic, and virologic diagnostic procedures. Given the presumed prevalence of swine TTVs and the hardy nature of Circoviridae in general, it appears that the porcine TTVs may fit the assumed characteristics of agent X. Once introduced into a PCV2-stable herd that is negative for agent X, it most likely acts as another infectious cofactor wherein the replication of PCV2 in tissues is upregulated and infectious PCV2 accumulates in tissues until PMWS is induced. In this context, agent X is biologically similar to other established infectious cofactors (ie, PPV, PRRSV, and *M hyopneumoniae*) as well as the cofactor effect of some vaccines.[17] Although it is premature and highly speculative to claim that the

identity of agent X is porcine TTV or TTV-like agents, the effects of disease facilitation mediated by g1-TTV described in the study reported here indicated that the TTVs are not the ubiquitous and harmless orphan viruses in search of a disease that many investigators have assumed.

a. Clone MFB-HB, Accurate Chemical & Scientific Corp, Westbury, NY.
b. Vectastain, Vector Laboratories, Burlingame, Calif.
c. Kirkegaard & Perry Laboratories Inc, Gaithersburg, Md.

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