Veterinary Microbiology, 133, 23-33

The emergence of multiple genotypes of PCV2, as demonstrated by phylogenetic analysis of whole genome or capsid sequences, makes it necessary to have quantitative diagnostic assay that Perform equally well on all strains. The objectives of this study were to develop and validate a novel real-time polymerase chain reaction (PCR) assay targeting the highly conserved rep gene (ORF1) and investigate the effects of diagnostic specimen choice on its performance. The assay was tested in naturally infected conventional pigs, experimentally infected gnotobiotic pigs, and plasmid-spiked negative serum, lung tissue, and feces and found to have a linear detection range of 2.2 x 10^3 to 2.2 x 10^10 copies of PCV2 per mL. The assay successfully detected and quantified PCV2 DNA in serum, buff coat, feces, and multiple lymphoid (bronchial, mesenteric, and superficial inguinal lymph nodes; thymus; tonsil; ileal Peyer's patches; and spleen), and non-lymphoid (myocardium lung; kidney; liver; and gluteal muscle) tissues from naturally infected pigs. Across all tissues and sera of naturally infected pigs, the mean PCV2 concentration was 3.0 logs higher in wasting versus non-wasting pigs. PCV2 concentration measured by tissue culture and immunohistochemical staining in homogenized liver samples of experimentally infected gnotobiotic pigs were compared to the concentrations estimated by quantitative PCR. Similar trends were noted with increasing PCV2 concentration detected in subclinically infected to severely PMWS-affected pigs across all assays. Our diagnostic assay was developed with a conserved target sequence, and performed efficiently in quantification of PCV2 in a variety of tissues from naturally and experimentally infected pigs. (C) 2008 Elsevier B.V. All rights reserved

Opriessnig, T., Patterson, A.R., Meng, X.J., Halbur, P.G. (2009) Porcine circovirus type 2 in muscle and bone marrow is infectious and transmissible to naive pigs by oral consumption
Veterinary Microbiology, 133, 54-64

Pork product, are a possible source of introduction of PCV2 isolates into a pig population. However, limited work has been done on the transmission through meat of porcine circovirus type 2 (PCV2), a Virus associated with several disease syndromes in pigs. The objectives of this study were to determine if pork products from PCV2-infected pigs contain PCV2 DNA/antig-en and to determine if the PCV2 present in the tissues is infectious by performing in vitro and in vivo studies. Skeletal muscle, bone marrow, and lymphoid tissues from pigs experimentally inoculated with PCV2 were collected 14 days post-inoculation (DPI). The tissues were tested for presence of PCV2 DNA by quantitative real-time PCR, for PCV2 antigen by immunohistochemistry (IHC), and for presence of infectious PCV2 by Virus isolation and inoculation of PCV2 naive pigs. Lymphoid tissues contained the highest amount of PCV2 (positive by PCR, IHC, and virus isolation), bone marrow contained a lower amount of PCV2 (positive by PCR and IHC but negative by virus isolation), and skeletal muscle contained the lowest amount of PCV2 (positive by PCR but negative by IHC and virus isolation). Naive pi's fed for three consecutive days with either skeletal muscle, bone marrow or lymphoid tissues all became PCV2 viremic as determined by
quantitative real-time PCR on serum starting, Lit 7 DPI. The pigs also seroconverted to PCV2 as determined by PCV2 IgM and IgG ELISA. In addition, PCV2 antigen was detected by IHC stains in lymphoid tissues and intestines collected from the majority of these pigs. Results from this study indicate that uncooked PCV2 DNA positive lymphoid tissues, bone marrow, and skeletal Muscle front PCV2 viremic pigs contain sufficient amount of infectious PCV2 to infect naive pigs by the oral route. (C) 2008 Elsevier B.V. All rights reserved

Hjulsager, C.K., Grau-Roma, L., Sibila, M., Enoe, C., Larsen, L., Segales, J. (2009) Inter-laboratory and inter-assay comparison on two real-time PCR techniques for quantification of PCV2 nucleic acid extracted from field samples Veterinary Microbiology, 133, 172-178

Several real-time PCR assays for quantification of PCV2 DNA (qPCR) have been described in the literature. and different in-house assays are being used by laboratories around the world. A general threshold of 10^6 copies of PCV2 per millilitre serum for postweaning multisystemic wasting syndrome (PMWS) diagnosis has been suggested. However, neither inter-laboratory nor inter-assay comparisons have been published so far. In the present study two different qPCR probe assays Used routinely in two laboratories were compared on DNA extracted From serum, nasal and rectal swabs. Results showed a significant linear association between the assays (p < 0.0001) and a systematic difference of 1.4 log(10) copies of PCV2 per millilitre of sample (p < 0.0001). This difference indicated that the assay from laboratory 1 yielded a higher output than the one from laboratory 2. Results also showed that there was no linear association between the amount of PCV2 DNA and the amount of total DNA, neither in nasal (p = 0.86) nor in rectal (p=0.78) swabs, suggesting that normalizing of PCV2 DNA load in swab samples to total DNA concentration is not suitable. The present exploratory study highlights the need for the performance of ring trials on qPCV2 protocols between laboratories. Meanwhile, the proposed thresholds for PMWS diagnosis should only be considered reliable for each particular laboratory and each particular assay. (C) 2008 Elsevier B.V. All rights reserved


Abstract not available


Objective - TO determine whether commercial Mycoplasma hyopneumoniae bacterins sold for use in swine contain porcine torque teno virus (TTV). Sample Population-22 commercially available M hyopneumoniae bacterins. Procedures-Direct and nested. PCR assays for genogroup-specific TTV DNAs were performed on serials of M hyopneumoniae bacterins by use of published and custom-designed primer pairs at 3 laboratories in North America and Europe. Results-Of the 22 bacterins tested by use of direct and nested PCR assays, 7 of 9 from the United States, 2 of 5 from Canada, and 4 of 8 from Europe contained genogroup 1 - and genogroup 2- TTV DNAs. In some bacterins, the TTV DNAs were readily detected by use of direct PCR assays. Conclusions and Clinical Relevance-Analysis of these data indicated that many of the commercially available M hyopneumoniae bacterins were contaminated with TTV DNA. It is possible that some of these bacterins could inadvertently transmit porcine TTV infection to TTV-naïve swine. (Am J Vet Res 2008;69:1601-1607)
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 post-weaning 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)

Objective-To determine whether porcine dermatitis and nephropathy syndrome (PDNS) could be experimentally induced in gnotobiotic swine. Sample Population-Plasma samples from 27 sows and 20 conventional weaned piglets were obtained, and 30 gnotobiotic pigs were used in experiments. Procedures-3 experiments were conducted. Groups of 3-day-old gnotobiotic pigs were inoculated with pooled plasma samples obtained from healthy feeder pigs in a herd that was in the initial phases of an outbreak of respiratory disease; gross and histologic lesions of PDNS were detected in the inoculated pigs. In a second experiment, 2- and 3-day-old gnotobiotic pigs were inoculated with porcine reproductive respiratory syndrome virus (PRRSV) and with PRRSV-negative tissue homogenate containing genogroup 1 torque teno virus (g1-TTV). Lesions of PDNS were detected. Results-Pigs inoculated with pooled plasma or the combination of tissue-culture-origin PRRSV and g1-TTV tissue homogenate developed systemic hemostatic defects, bilaterally symmetric cutaneous hemorrhages, generalized edema, icterus, bilaterally symmetric renal cortical hemorrhage, dermal vasculitis with hemorrhage, and interstitial pneumonia consistent with a clinical and pathologic diagnosis of PDNS. The PRRSV RNAs and g1-TTV DNAs were detected in plasma; all pigs seroconverted to PRRSV, and all had negative results for porcine circovirus type 2 when tested by use of PCR assays. Conclusions and Clinical Relevance-These data suggested that PDNS is a manifestation of disseminated intravascular coagulation in swine. For the experimental conditions reported here, combined infection with g1-TTV and PRRSV was implicated in the genesis of these lesions. (Am J Vet Res 2008;69:1615-1622)