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Phylogenetic characterization of the first Ungulate tetraparvovirus 2 detected in pigs in Brazil

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ABSTRACT

Ungulate tetraparvovirus 2 (UTV2), formerly known as porcine hokovirus due to its discovery in Hong Kong, is closely related to a *Primate tetraparvovirus* (human PARV-4) and *Ungulate tetraparvovirus 1* (bovine hokovirus). Until now, UTV2 was detected in European, Asian and North American countries, but its occurrence in Latin America is still unknown. This study describes the first report of UTV2 in Brazil, as well as its phylogenetic characterization. Tissue samples (lymph node, lung, liver, spleen and kidney) of 240 piglets from eight different herds (30 animals each herd) were processed for DNA extraction. UTV2 DNA was detected by PCR and the entire VP1/VP2 gene was sequenced for phylogenetic analysis. All pigs from this study displayed postweaning multisystemic wasting syndrome (PMWS). UTV2 was detected in 55.3% of the samples distributed in the variety of porcine tissues investigated, as well as detected in almost all herds, with one exception. The phylogenetic analysis demonstrated that Brazilian UTV2 sequences were more closely related to sequences from Europe and United States.

Introduction

Porcine hokovirus, recently classified as *Ungulate tetraparvovirus 2* (UTV2) belongs to the *Parvoviridae* family and is related to *Primate tetraparvovirus* (formerly known as human PARV-4) and *Ungulate tetraparvovirus 1*(UTV1) (formerly known as bovine hokovirus).[1,2] The UTV2 genome organization contains two major open reading frames (ORFs), named ORF1 and ORF2, separated by a small non-coding region. ORF1 encodes non-structural proteins (NS) and ORF2 encodes the two overlapping capsid proteins (VP1/VP2) and a small conserved putative protein.[1] Studies demonstrated high prevalence rates of UTV2 in domestic pigs and wild boar populations ranging from 22.8% to 50.5%.[1,3,4] UTV2 can infect pigs of different ages but higher prevalence was found in

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older pigs (grow-finishing),[5] as well as in adult wild boars.[3] UTV2 has been detected in domestic pigs in many countries as Hong Kong,[1] United States,[5] China,[6] Cameroon,[7] as well as wild boars from Germany[3] and Romania,[4] indicating that the virus can be endemic worldwide in both domestic and wild pig population.

UTV2 has been detected in a variety of porcine tissues, fecal and serum samples from healthy or sick pigs, but no clinical symptoms have been linked to infection or viral persistence.[1,3,8] However, one study observed that pigs displaying respiratory disease had higher levels of UTV2 compared to those with enteric disease or abortion.[5] In addition, some studies demonstrated concurrent infection between emerging parvovirus with the porcine circovirus 2 (PCV2).[9,10] Therefore, the aim of this study was to detect and analyze phylogenetically UTV2 in pigs suffering from postweaning multisystemic wasting syndrome (PMWS).

Material and methods

Samples

In 2005, during a PMWS outbreak, tissue samples were submitted to the Department of Pathology at Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil, for PMWS diagnosis and research purposes. The samples (lymph node, lung, spleen, liver and kidney) were collected from 240 animals, approximately 12–15 weeks of age, from eight different herds (30 animals each herd) from Rio Grande do Sul State, Brazil. The inclusion criteria adopted to fulfill the triad of requisites to define PMWS disease was followed as proposed by Sorden (2000)[11]: (i) presence of clinical signs, such as lessened weight gain, diarrhea and wasting; (ii) histopathological evidence of lymphocytic depletion, multinucleated giant cell formation in lymph nodes, histiocytic replacement of follicles in lymphoid tissues, and multifocal lymphohistiocytic interstitial pneumonia and positive presence of PCV2 antigen in the lungs by immunohistochemistry (IHC) associated with microscopic lesions; (iii) PCV2 DNA confirmation by PCR. Sections of swine lymph nodes and lungs were submitted for histopathological investigation consisting of the hematoxylin–eosin standard treatment, with samples embedded in paraffin blocks. To identify PCV2 antigen in the lesion site, sections of the organs presenting pathological changes were submitted to IHC analysis using the anti-PCV2 polyclonal antibody (Department of Veterinary Diagnostic and Production Animal Medicine, Ames, IA, USA) diluted to 1:1000, followed by streptavidin–biotin–peroxidase complex technique (Dako Cytomation Inc., Carpinteria, CA, USA), and revealed using diaminobenzidine method (Dako Cytomation Inc., Carpinteria, CA, USA).

Tissue samples (approximately 1 g) were minced and homogenized in 1 mL of phosphate buffered saline (PBS, pH 7.4). The homogenized tissues were three times frozen and thawed, and then clarified by centrifugation at 6160 × *g*. Total nucleic acids were extracted from 100 μL of each tissue homogenate, using a commercial extraction kit based on silica (NewGene kit, Simbios Biotecnologia, Brazil) as recommended by the manufacturer.

PCR detection and sequencing

PCV2 was detected using a previously described PCR protocol.[12] Primers for UTV2 were selected using the Vector NTI Advance 10 Software (sense: 5′-GTG GCA GTG ATA TTG CAT CG-3′ and antisense: 5′-TGG CAG TCA TTG AAT GGA AA-3′). PCR mix conditions were the following: 2.5 μL of 10× buffer, 1.5 mM of $MgCl_2$, 200 μM of dNTPs, 20 pmol of each primer, 1 U of *Taq* DNA polymerase (Ludwig Biotecnologia, Brazil), 2 μL of DNA sample and water up to 25 μL. PCR thermal cycle was performed with an initial cycle of 95 °C for 2 min, 35 cycles at 95 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min and a final extension at 72 °C for 5 min, which amplified a product of 250 base pairs (bp). For the phylogenetic analysis, primers were selected in order to cover the entire VP gene using Vector NTI Advance 10 software (Table 1). PCR mix and thermal conditions for each PCR were the same as described above. The sequences were randomly selected from distinct herds and the amplified DNA samples (30–45 ng) were purified with NucleoSpin® II kit, (Macherey-Nagel, Germany), labeled with 3.2 pmol of each primer (Table 1) and 2 μL of BigDye Terminator v3.1 Cycle Sequencing RR-100 (Applied Biosystems, USA) using the automatic sequencer ABI-PRISM 3100 Genetic Analyzer, armed with 50 cm capillaries and POP6 polymer (Applied Biosystems, USA).

Phylogenetic analysis

The VP data set was composed by assembled sequences (DNA Baser version 3.0 Software) from this study (JQ700067–JQ700072) and sequences retrieved from Genbank EU200671–EU200677, JN990266–JN990269 from China, FJ982246–FJ982255 from Great Britain, JQ425257–JQ425259 from United States, JF738350, JF738351, JF738357, JF738362, JF738364, JF738366–JF738368 from Romania and GQ869539, GQ869540, GQ869542, GQ869543 from Germany. An UTV1 sequence (EU200668) was used as outgroup in the phylogenetic analysis. The VP data set was aligned using MEGA Software version 4.0.[13] The phylogenetic analysis was performed using two methods: a heuristic search using PAUP 4.0.b software[14] with a support of 1000 bootstrap repetitions and a Bayesian Inference (BI) was conducted with MrBayes 3.1.2 software.[15] The substitution models for these approaches were found using MrMODELTEST with the AIC criterion. For the BI method, four Markov chains, one cold and three heated were used and the run was set for 2×10^6 generations, with trees sampled every 100 generations. Trees generated prior to stationary phase were discarded as "burn-in".

Results

All pigs from the present study fulfilled the requisites for the case definition of PMWS as previously proposed by Sorden.[11] In the histopathology analysis, all of the lymph node samples displayed lymphocytic depletion, multinucleated giant cell formation and histiocytic replacement of follicles. In addition, the lung samples demonstrated multifocal lymphohistiocytic interstitial pneumonia. PCV2 antigen was detected by IHC in all lung and lymph node samples and all the samples were