

# Porcine Viruses Not Previously Reported in Feral Swine in Florida

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**ABSTRACT:** Although national surveillance is conducted throughout the United States for multiple pathogens associated with feral swine, many pathogens that persist in wild pigs globally have not been the subject of investigations within the U.S. We surveyed feral swine in Florida for two viruses that are ubiquitous in domestic and wild pigs in Europe: torque teno sus virus type 1 and porcine lymphotropic herpesvirus. We found these viruses to be prevalent in feral swine in Florida. We detected viral DNA in nasal and genital swabs or whole blood collected from animals at multiple locations throughout the state. Our results suggest that not only are animals infected with these viruses, but that they are actively shedding virus and capable of transmitting them at multiple, geographically disparate locations. These viruses have the potential to be pathogenic when an animal is coinfecting with other porcine viruses, and these viruses pose a potential threat to other wildlife, livestock, and people.

**KEY WORDS:** anellovirus, disease, Florida, gammaherpesvirus, pig viruses, porcine lymphotropic herpesvirus, *Sus scrofa*, torque teno virus

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## INTRODUCTION

Feral swine (*Sus scrofa*) harbor multiple pathogens that are important to human, livestock, and wildlife health. In the United States, feral swine are regularly found to be antibody positive for such livestock pathogens as *Brucella suis*, *Mycobacterium* spp., *Coxiella burnetii*, porcine reproductive and respiratory syndrome virus, porcine circovirus type 2, swine influenza virus, and pseudorabies virus (Corn et al. 2009, Wyckoff et al. 2009, Stephenson et al. 2015). Some of these pathogens, such as swine influenza virus, are known human pathogens (Saliki et al. 1998) while others, such as pseudorabies, cause mortalities in native wildlife and livestock (Caruso et al. 2014). Other pig-associated pathogens, like porcine epidemic diarrhea virus, cost domestic livestock producers millions of dollars each year, while some foreign animal diseases remain a threat to the livestock industry in this country (e.g., classical swine fever; USDA 2015). While these particular pathogens pose a threat to food security and global public health, other microbes are known to occur in wild pigs but are much less frequently studied.

Gammaherpesviruses belong to a large and diverse group of herpesviruses known to infect wild and domestic ruminants. In healthy individuals these viruses remain latent, but stress and immunosuppression reactivate the viruses, which can cause a variety of mutagenic diseases (Ackermann 2006), particularly following transplantation of infected tissue into a new host (Huang et al. 2001). Three types of porcine lymphotropic herpesviruses (PLHVs: PHLV-type 1, PLHV-type 2 and PLHV-type 3) have been described in wild pigs from Europe (Ehlers et al. 1999, McMahon et al. 2006), but there are few reports on their prevalence or distribution in feral swine in North America.

Likewise, anelloviruses are ubiquitous viruses found in a large number of vertebrates, suggesting co-evolution with their hosts (Bendinelli et al. 2001). They cause disease and death in immunocompromised individuals, particularly in mammalian hosts co-infected with other viruses (Gergely et al. 2005, Huang et al. 2010). Both

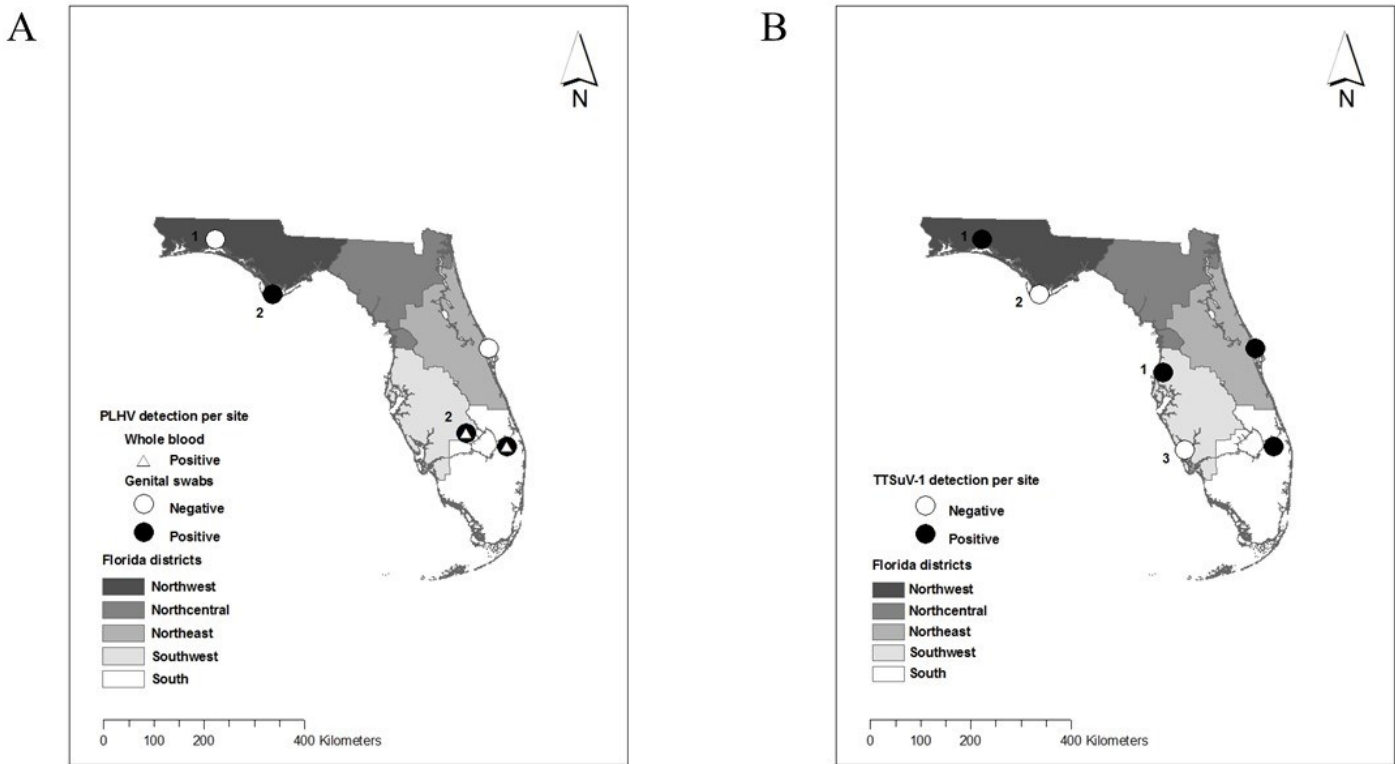
torque teno sus virus (TTSuV) and PLHV are associated with pathology in pigs during coinfection with other porcine viruses (Huang et al. 2010) and may play a role in post-weaning multisystemic wasting syndrome (McMahon et al. 2006, Aramouni et al. 2011, Vlasakova et al. 2014), a major source of morbidity in domestic swine. There are two closely related but distinct groups of TTSuV: TTSuV-type 1 and TTSuV-type 2, both of which are thought to have a global distribution in wild and domestic pigs (McKeown et al. 2004).

Neither PLHV nor TTSuV prevalence has been characterized in feral swine in the U.S. The purpose of this study was to determine the prevalence of viral infection in feral swine in selected locations in Florida.

## METHODS

From 2014 to 2015, biological samples were collected from 62 feral swine at seven sites in Florida. Animals were opportunistically collected as part of a national feral swine disease monitoring effort led by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Disease Program. Feral swine were either trapped and euthanized during animal control efforts, or shot by hunters in federal and state wildlife management areas, military bases, and private properties year-round. Sites were chosen to represent the five Florida Fish and Wildlife Commission's Wildlife Management Districts, and each site had a minimum of six pigs available for sampling per site (Figure 1). Six sites were sampled for TTSuV and five sites were sampled for PLHV. Four of the sites were surveyed for both viruses: two in the northwestern region, one in the northeast, and one in the south.

In order to determine the predominant type of PLHV, we tested for the presence of all three PLHVs in blood samples collected from two sites. Blood was screened because these viruses are associated with infection of B-cells (Huang et al. 2001). Preliminary data from the two sites in the South and Southwest Districts (Figure 1) identified only PLHV-type 2, and for the rest of the samples



**Figure 1. Sites in Florida where feral swine were trapped between 2014-2015 and screened for evidence of infection with porcine lymphotropic herpesviruses (PLHVs; Figure 1A) and/or torque teno sus virus-type 1 (Figure 1B) using molecular methods. Sites where no positive pigs were identified are indicated with an open circle. Figure 1A: sites where PLHV-positive pigs were identified are indicated by a black circle (genital swabs) or white triangle (whole blood), respectively. Figure 1B: sites where TTSuV-1-positive pigs were identified are indicated by a black circle.**

and sites, we screened genital swabs for the presence of PLHV-type 2 in order to explore the hypothesis that vertical transmission from mother to offspring occurs (Tucker et al. 2003). Genital swabs were collected from females only. Only nasal swab specimens were screened for TTSuV-type 1, as an oral-nasal route of transmission has been reported for this virus (Cortney et al. 2012).

We extracted DNA from nasal, genital and blood samples using a commercial kit (DNeasy Blood and Tissue Kit, Qiagen, Valencia, CA). Following extraction, we used polymerase chain reaction (PCR) assays to amplify the viral DNA and used Sanger sequencing (Wilson and Walker 2005, Valones et al. 2009) to confirm identity of the viruses. For PCR assays that amplified all PLHVs from whole blood specimens, we used primers capable of amplifying a segment of the DNA polymerase gene conserved among *Herpesviridae*, resulting in a 192-nucleotide basepair (bp) product, as previously described (VanDevanter et al. 1996). For PCR assays that amplified only PLHV-type 2, we designed an assay to amplify a segment of the glycoprotein B gene, resulting in a 440-nucleotide basepair (bp) product. This assay consisted of a 50 microliter reaction, with 600nM of the forward primer (PLHV2\_FOR: 5'-TAGGCGGCTTGCTCTGGCAC-3') and 600nM of the reverse primer (PLHV\_2REV: 5'-CAGTTTCCAAGGTTTCGATGGAC-3'), 25 microliters of Promega Ready-to-Go master mix (Promega, Madison, WI), and five microliters of DNA extracted from genital

swabs as template. The following thermogenic profile was used for this assay: 95°C for two minutes followed by 40 cycles of 95°C for 30 seconds, 59° for one minute, and 72° for one minute, with a final extension of 72° for seven minutes. For the PCR assay that amplified TTSuV-type 1 from nasal swab specimens, we used primers capable of amplifying a segment of the open reading frame (ORF) 2 of this virus resulting in a 678-nucleotide basepair (bp) product, as previously described (Cortney et al. 2012).

Resultant DNA amplified from PCR was column purified and bi-directionally sequenced at the University of Florida Interdisciplinary Center for Biotechnology Research using dideoxy Sanger sequencing. Consensus nucleotide sequences were determined using chromatograms generated from bi-directional Sanger sequencing using Geneious software version 2.6.2 (Biomatters Ltd, Auckland, New Zealand). Chromatogram discrepancies among sequencing directions were resolved by-hand and in Geneious (Luca Curci and Sonnante 2015). The final sequences were compared to published sequences using the basic local alignment search tool in the software BLAST (Altschul et al. 1990) in order to confirm identity of the viral DNA. Animals were considered positive for a sample if the sequence was identified as one of the two viruses and negative for the viruses if no viral DNA was amplified in the PCR assay. We estimated prevalence of infection of PLHV in feral swine at five sites and TTSuV-type 1 at six sites in Florida.

## RESULTS

Using DNA extracted as from whole blood as template, PLHV-type 2 DNA was identified in 48.1% (13/27) of pig samples collected from two field sites in south central part of the state (Table 1, Figure 1A). Prevalence of the virus in feral swine ranged from 45-60% between the two sites ( $54.5 \pm 7.5\%$ ; mean  $\pm$  SE). Using DNA extracted from genital swabs as template, PLHV-type 2 DNA was detected in 23.5% (8/34) of pig samples collected from five field sites in Florida (Table 1, Figure 1A). Prevalence of the virus in feral swine ranged from 0-67% per site ( $20 \pm 12.4\%$ ). Amplicon sequencing revealed homogeneity among all individuals; we found that PLHV-type 2 glycoprotein B sequences were identical among individuals at geographically separated sites.

Using DNA extracted from nasal swabs as template, TTSuV-type 1 DNA was detected in 25.0% (9/36) of pig samples collected from six field sites in Florida (Table 1, Figure 1B). Prevalence of the virus in feral swine ranged from 0-67% per site ( $25 \pm 10.3\%$ ). Sequences generated in this study were 97-99% identical to published sequences (GenBank Accession numbers JF451512, JF451485, JF451513 & JF451479). We found that TTSuV-type 1 sequences generated in this study were heterogeneous in feral swine. TTSuV-type 1 was genetically variable both within sites and between sites, suggesting that the virus is highly mutable and may contain phylogenetic signal useful for studies of host population dynamics. Sequence analysis of TTSuV-type 1 amplicons revealed variation of 2-3% between individuals from the same site and up to 5-8% between disparate sites.

## DISCUSSION

In this study, PLHV-type 2 and TTSuV-type 1 prevalence and distribution were explored in Florida for the first time. Few studies exist on the prevalence of PLHV in either domestic or wild pigs in North America. In Ireland, 74% of domestic pigs on 22 farms were infected with PLHV-type 1 (McMahon et al. 2006). In our study, positive samples were detected in pigs at three of the five sites sampled for this virus (Figure 1). However, the ideal sampling type (lymphoid tissues or peripheral blood mononuclear cells) was not collected, and thus the true prevalence could be higher than reported in this study. Evidence of PLHV-2 DNA in vaginal swabs at two of the four sites supports the hypothesis of vertical transmission of the virus, which has been suggested in previous studies (Tucker et al. 2003), or alternatively suggests the possibility of sexual transmission of the virus, which has been demonstrated for other gammaherpesviruses (Hussy et al. 2002). While high levels of genetic variation were found in TTSuV-type 1, we found no variation in PLHV-type 2 either within or among populations, suggesting that lymphotropic herpesviruses are stable within their hosts. The higher variation found in TTSuV-type 1 suggests that it is more mutable and has the potential to serve as a model system for more pathogenic organisms (especially single stranded DNA and RNA viruses) that may be shared among or between domestic and feral pigs.

Only a handful of studies have been conducted on TTSuV in North America. A previous study found TTSuV-

**Table 1. Prevalence of porcine lymphotropic herpesvirus-type 2 (PLHV-2) and torque teno sus virus-type 1 (TTSuV-1) at seven sites in Florida. Prevalence was measured as the number of individuals that tested positive for the virus, divided by the total number of samples tested. For PLHV-2, 46 unique individuals were sampled. Fifteen individuals had both blood and nasal sample types; the rest had one or the other. For TTSuV-1, 36 unique individuals were sampled for the virus.**

Target Organism	Location by Region	Sample Type	Prevalence (%)
PLHV-2	South	Whole blood	3/5 (60%)
PLHV-2	Southwest #2	Whole blood	10/22 (45%)
<b>Prevalence in whole blood samples</b>			<b>13/27 (48.1%)</b>
PLHV-2	South	Genital swab	2/6 (33%)
PLHV-2	Northwest #2	Genital swab	2/10 (20%)
PLHV-2	Southwest #2	Genital swab	4/6 (67%)
PLHV-2	Northeast	Genital swab	0/6 (0%)
PLHV-2	Northwest #1	Genital swab	0/6 (0%)
<b>Prevalence in genital swab samples</b>			<b>8/34 (23.54%)</b>
<b>Prevalence in individuals</b>			<b>15/46 (32.6%)</b>
TTSuV-1	Southwest #1	Nasal swab	1/6 (17%)
TTSuV-1	Northeast	Nasal swab	2/6 (33%)
TTSuV-1	South	Nasal swab	2/6 (33%)
TTSuV-1	Northwest #1	Nasal swab	4/6 (67%)
TTSuV-1	Northwest #2	Nasal swab	0/6 (0%)
TTSuV-1	Southwest #3	Nasal swab	0/6 (0%)
<b>Prevalence in nasal swab samples</b>			<b>9/36 (25.0%)</b>

type 1 to be highly prevalent in domestic pig herds from Quebec, Canada (Brassard et al. 2008). In that study, 81% of animals tested positive by PCR using whole blood samples, and 60% of fecal samples tested positive. Here, we tested nasal swabs of feral swine using PCR and found that nearly a quarter of feral swine sampled were actively shedding viral DNA via the nasal mucosa. As expected, the prevalence of TTSuV in free-ranging feral swine was lower than in domestic herds, as production animals typically have higher rates of infection compared to free-ranging animals due to the high density living conditions and frequent movement among farms (Brassard et al. 2013). Our study illustrates that nasal swabs are useful for the detection of TTSuV in feral pigs and supports the hypothesis that transmission occurs via the oral-nasal route (Cortney et al. 2012). To our knowledge, this is the first study to identify TTSuV-1 DNA in porcine nasal secretions in North America.

The discovery of viruses circulating in Florida that have a high prevalence but a low relative pathogenicity is noteworthy. Both viruses have the capacity to cause disease when there are coinfections or in immunocompromised individuals (Huang et al. 2010, Aramouni et al. 2011). Thus, these pathogens may add to the disease burden of feral swine in Florida.

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