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Potential for the cross-species transmission of swine torque teno viruses

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ABSTRACT

Torque teno viruses [TTVs] are negative sense, single-stranded, DNA viruses, which are distributed globally in several mammalian hosts such as humans, apes, sheep and swine in a species-specific manner. While the pathogenic potential of TTVs is under debate, recent experimental studies in gnotobiotic pigs indicate that swine TTVs, TTSuV1 in particular, can act as a primary or co-infecting pathogen. Hence, determining whether TTSuV1 can infect other mammals would eventually further our understanding of viral pathogenesis, especially in co-infections. In this study, we tested sera from horses, cattle, sheep, dogs and elk for the presence of TTSuV1 DNA using a panel of TTSuV1-specific primers, and assessed the extent of sero-conversion to TTSuV1 in the selected species. We found that TTSuV1 DNA was detected in 46.7% of equines, 70% of canine, 100% of bovine, 40% of ovine and 93.3% of elk samples. However, significant TTSuV1 specific antibody responses were detected only in the bovine, ovine and equine samples but not the canine or elk samples, indicating that these animals could support the replication of TTSuV1. This combined serological and molecular epidemiological profile of TTSuV1 infection in five different species indicates the host range of species-specific TTVs could be wider than initially believed. Further studies are required to understand the health risks to these animal species from TTSuV-1 infection.

1. Introduction

Torque teno viruses (TTV) were first discovered in 1997 in serum of a human patient with post-transfusion hepatitis (Nishizawa et al., 1997). Since then species-specific TTVs were discovered in a wide range of hosts including sea lions, tupaias, cattle, chimpanzees, cats, dogs, sheep, camels and chickens (Okamoto, 2009b). TTVs are non-enveloped, small, circular single-stranded DNA viruses in the *Anelloviridae* family (Huang et al., 2010; Okamoto, 2009c). Swine-origin, Torque teno sus viruses (TTSuV) consist of two major genogroups, TTSuV1 and TTSuV2, which differ by about 40–50% sequence identity (Niel et al., 2005).

Torque teno viruses are ubiquitous environmental contaminants and are found in water sources, sewage and air (D'Arcy et al., 2014; Griffin et al., 2008). They have also been detected in human drugs, pork products and veterinary vaccines (Kekarainen et al., 2009; Leblanc et al., 2014). While extensive epidemiological evidence for the association of human TTVs with various disease conditions including respiratory, nervous and auto-immune illnesses is available (Okamoto, 2009a), there are very few studies on the mechanistic basis of possible pathogenesis. Hence, the pathogenicity of human TTVs is still under debate. Swine TTVs are found in both diseased and healthy domestic pigs. However, the rate detection of TTSuV's in pigs which manifest clinical signs of the coinfecting pathogens is roughly twice that of clinically healthy pigs (Rammohan et al., 2012). In experimental studies on gnotobiotic pigs, TTSuV1 induced porcine dermatitis and nephropathy syndrome (PDNS) and exacerbated post-weaning multi-systemic wasting syndrome [PMWS] (Ellis et al., 2008; Krakowka and Ellis, 2008). Since pork consumption is common and there is an increasing interest in using swine for xenotransplantation, we examined the potential transmission of TTSuV1 to humans in a previous study (Ssemadaali et al., 2016). We found TTSuV1 DNA is widely detectable in human sera and human peripheral blood mononuclear cells (PBMC's) support the replication of TTSuV1. In this study, we have tested the hypothesis that TTSuV's can also potentially infect other domestic and wild animals. We tested sera from equine, bovine, ovine, canine and elk species for the presence of TTSuV1 DNA, as well as sero-conversion to TTSuV1. We detected TTSuV1 DNA in all species tested. However, only equine, bovine and ovine species showed clear evidence of sero-conversion. Our data suggests the host-range of species-specific TTVs, particularly TTSuV1, could be wider than initially thought. Our findings have important implications for better understanding the transmission of TTSuV1 and torque teno viruses as members of host viromes.

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2. Materials and methods

2.1. Samples for analysis

A total of 232 sera from five different species were used for analysis, namely elk (N = 50), bovine (N = 50), equine (N = 50), ovine (N = 50) and canine (N = 32). All 232 samples were from case submissions to the North Dakota State University [NDSU] Veterinary Diagnostic Laboratory. Samples selection was blinded to case history or location. All experimentation was carried out in compliance with the Institutional Review Board [IRB] and Institutional Biosafety Committee [IBC] policies of NDSU.

2.2. Analysis of sera by a TTSuV1-specific ELISA

All samples were analyzed in duplicate with two experimental replicates (total of 4 values) with a TTSuV1-specific ELISA, essentially as described before (Ssemadaali et al., 2016). Briefly, 96-microwell ELISA plates (Thermo Fisher, Waltham, MA) were coated with 50 µl of a recombinant TTSuV1 ORF2 antigen (1 mg/ml) per well at a dilution of 1:100000 in carbonate coating buffer (pH 9.6). After an overnight incubation at room temperature, the plates were washed five times using phosphate buffered saline with tween [PBST], and blocked with 5% BSA in General Block [Immunochemistry, Bloomington, MN] at a volume of 100 µl per well for 2 h at 37 °C. After the blocking, plates were washed five times using PBST. Each serum was diluted at 1:50 in PBST, added in 50 µl volumes to the plate and incubated for 2 h at 37 °C, followed by washing five times with PBST. A 50 µl volume of a 1:2500 diluted species- specific peroxidase-conjugated secondary antibody (KPL, Gaithersburg, MD) was added to each well and the plates were incubated at 37 °C for 1hr. For the detection of TTSuV1-specific antibodies in the elk samples, an anti-deer conjugate was used as previously described (Colby et al., 2002). After five additional washing steps, the peroxidase reaction was catalyzed by using a tetramethylbenzidine [TMB] -hydrogen peroxide solution as a substrate (KPL, Gaithersburg, MD) and incubated for 15mins at room temperature in the dark. Finally, the reaction was stopped by adding 50 µl of 1 M HCl to each well. The optical density [OD] readings were obtained at 450 nm using a spectrophotometer.

2.3. PCR detection of TTSuV1 DNA

Fifteen samples from each species, consisting of five samples each with high, medium and low ODs on the ELISA were selected for PCR. Of the 32 canine samples, 10 were selected for PCR analysis. Total DNA was extracted from the selected samples using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA), following the manufacturer's instructions. Samples were analyzed for the presence of TTSuV1 DNA by three different assays, at a minimum in duplicates. The first assay [designated TTSuV-UTR-qPCR] is a probe- based Taq Man assay targeting the untranslated region [UTR] region (Xiao et al., 2012). The second and third assays, targeted the TTSuV1 ORF2 or ORF3 (designated TTSuV1 ORF2 or ORF3 PCRs). The UTR region is highly conserved and commonly used for PCR detection of TTVs. The ORF2 and 3 are more variable and hence, more strain specific. The genomic regions targeted by PCR are denoted in Fig. 1. All PCRs were previously validated and conducted essentially as described before (Ssemadaali et al., 2016). The primers for the amplification of the ORF2 segment consisted of 5′agtcaagcttttgccggaacactgggaggaag3′ and 5′acgtctcgagccagccatcgtcgccgat3, while primers for the detection of the TTSuV1 ORF3 consisted of 5′gcgacgatggctgtttggaggtgaaataccaaccc3′ and 5′acgtctcgaggcgtttctttgttttttat3′. Both assays were optimized at 25 ng of template DNA in a commercial PCR master mix (ReadyMix™ Taq PCR Reaction Mix, Sigma, St. Louis, MO), and a Tm of 56 °C for 35 cycles. The TTSuV1 ORF2 PCR amplifies a 235 bp product while the TTsuV1 ORF3 PCR amplifies a 477 bp product. No template and a positive control samples were included in each run.

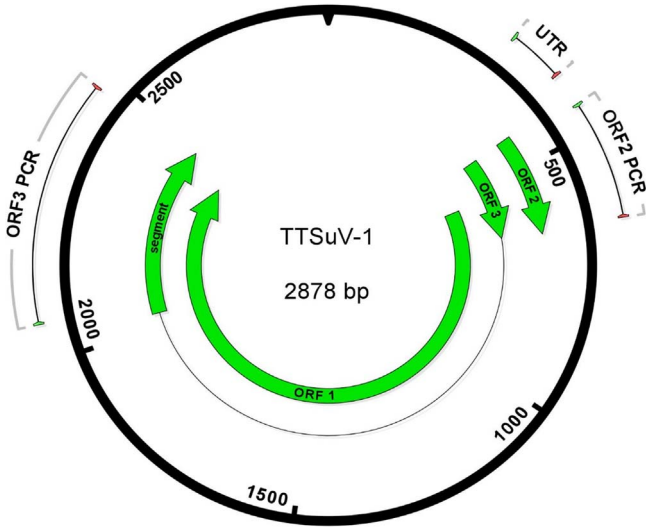

Fig. 1. Diagram of PCRs used for TTSuV1 detection: The TTSuV1 genome showing the UTR and open reading frames. The TTSuV1 qPCR or PCR1 targeted the UTR, TTSuV1 ORF2 PCR or PCR2 targeted the ORF2 and the TTSuV1 ORF3 PCR or PCR3 targeted the ORF3.

2.4. PCR detection of human TTV DNA

Total DNA was extracted from the selected samples as described above. A previously published protocol (Okamoto et al., 2001) for the universal detection of human TTV DNA was adapted to a real-time PCR format as previously described (Ssemadaali et al., 2016). Briefly, 5 µl of template DNA, primers 5′gtaagtgcacttccgaatggctgag3 and 5′gcccgaattgcccttgac3′ were combined with a probe 5′ - FAM - TTTTC CACGCCCGTCCGCAG - BHQ-1 - 3′ at 0.2 µM primer, 0.1 µM probe concentrations in QuantiFast Probe PCR Kit (Qiagen, Valencia, CA). Cycling conditions included a Tm of 65 °C for 40 cycles. No template and positive control samples were included in each run.

2.5 Sequencing and phylogenetic analysis

Although previously validated (Ssemadaali et al., 2016), amplified PCR products were selected at random for each species, from each of the three PCR reactions, and sequenced. The identity of the amplicon was confirmed using the BLAST tool (National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM)). To determine if any distinctive clustering of TTSuV1 sequences from each species occurred, phylogenetic analysis of the sequenced amplicons was carried out using the MegAlign software (DNASTAR Inc., Madison, WI) of the LaserGene core suite 11.0. Representative TTSuV1, TTSuV2 or human TTV UTR, ORF1 or 2 sequences were downloaded from GenBank and sequence alignment carried out with the CLUSTAL W program. Rooted phylogenetic trees were generated by the neighbor-joining method. Corresponding human TTV sequences were used as the "outgroup".

3. Results

3.1. TTSuV1 DNA was widely detected in sera of domestic and wild animals

Of the 15 equine samples examined, seven [46.7%] were positive by the PCR targeting the UTR, while 3 and 1 samples were positive for the ORF2 and ORF3 PCR's respectively [Fig. 2]. Thirteen of the 15 bovine samples tested were positive by the UTR PCR and ORF2 PCRs, while only one sample was negative for the ORF3 PCR [Fig. 2B]. Six of the 15 [40%] ovine samples were positive for the UTR PCR, while 2 samples were positive for the ORF2 PCR and none for the ORF3 PCR. While

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