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Molecular detection of Torque teno sus virus in lymphoid tissues in concomitant infections with other porcine viral pathogens

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ABSTRACT

In this study, 40 pigs with respiratory and wasting disorders from Cuban swine herds were screened by PCR for the presence of TTSuV1, TTSuV2, PCV-2, PPV and CSFV in spleen samples. The variability of the porcine TTSuV sequences obtained was investigated by phylogenetic analysis. This study showed for the first time that TTSuV1 and TTSuV2 were present in Cuban swine herds. The investigation revealed the following infection rates: TTSuV1 40%, TTSuV2 37.5%, PCV-2 70%, PPV 37.5% and CSFV in 52.5%. The presence of two or more of these viruses at different rates in the same spleen samples was revealed. Also, a higher genetic diversity of TTSuV2 sequences was observed regarding TTSuV1 sequences.

1. Introduction

Torque teno virus (TTV), was discovered in Japan in 1997 in a patient with post-transfusion hepatitis of unknown aetiology (Nishizawa et al., 1997). Since then, a large number of TTV strains infecting humans, swine, poultry, cattle, sheep, cats and dogs have been identified (Okamoto et al., 2002; Okamoto, 2009).

TTVs are small, non-enveloped circular single-stranded DNA viruses classified into the family *Anelloviridae* by the International Committee on Taxonomy of Viruses (ICTV).

In recent years, TTVs have attracted considerable interest within the research community (Kekarainen and Segalés, 2009) and porcine TTV infections have been shown to be widespread in pigs from different countries including the United States, Canada, Spain, China, Korea, Thailand, Japan, Brazil, Germany and Hungary (McKeown et al., 2004; Okamoto et al., 2002; Niel et al., 2005; Gallei et al., 2010; Takács et al., 2008). The genome of porcine TTV is approximately 2.8 kb and studies in swine have identified two distinct TTV genogroups: TTV-1 and TTV-2 (Niel et al., 2005). Recently, both genogroups have been defined as species (Huang et al., 2010).

Despite the fact that TTV infection in humans is not considered to be directly associated with a disease (Jelcic et al., 2004), porcine TTV has been proven to partially contribute to the experimental induction of porcine dermatitis and nephropathy syndrome (PDNS) combined with the porcine reproductive and respiratory syndrome virus (PRRSV) infection (Krakowka et al., 2008), and also to the experimental induction of postweaning multisystemic wasting syndrome (PMWS) combined with PCV-2 infection in a gnotobiotic pig model (Ellis et al., 2008). Moreover, high rates of TTSuV, especially TTSuV2, have been detected in PMWS pigs (Kekarainen et al., 2006). These results suggest that porcine TTV is probably pathogenic in pigs due to synergistic effects of different viruses acting together. However, further studies will be required to associate TTV infection with specific diseases.

The aim of this study was to detect the presence of TTSuV1 and TTSuV2 in Cuban swine herds and their possible association with other porcine viruses.

Samples were collected from 40 pigs in ten different swine herds located in eight Cuban provinces in 2005 and 2009 (Table 1). The selected animals showed a variety of clinical signs that included respiratory and wasting disorders. Total DNA and RNA were extracted using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) and TRI Reagent (LS, SIGMA, San Louis, Missouri, USA), respectively. PCR and RT-PCR assays were performed to detect the possible presence of TTSuV1/TTSuV2, porcine circovirus 2 (PCV-2), porcine parvovirus (PPV) and classical swine fever virus (CSFV) genome as described previously by Segalés et al. (2009), Sandvik et al. (2001), Kim et al. (2003) and Díaz de Arce et al. (1998), respectively.

A partial sequence (250 bases) of the non-coding region of swine TTV, proposed by Segalés et al. (2009) was carried out. The estimation of evolutionary divergence was conducted using the

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Table 1
Spleen samples assessed by PCR assays for PCV-2, TTV-1, TTV-2, PPV and CSFV detection.

Province	Sample code	PCV-2	TTV-1/A. Number	TTV-2/A. Number	PPV	CSFV
Pinar del Río	PR1_2007	+	+/FN687862	–	+	+
	PR2_2007	+	–	–	+	+
	PR3_2007	+	–	–	+	+
	PR1_2008	+	–	–	+	–
	PR1_2009	+	+/FN687863	+/FN687880	+	–
La Habana	HA1_2007	–	–	+/n.s	–	+
	HA2_2007	+	–	–	+	–
	HA3_2007	+	–	–	+	+
	HA4_2007	–	–	–	+	–
	HA5_2007	–	+/n.s	–	–	–
	HA6_2007	–	–	–	–	–
	HA7_2007	–	–	–	–	–
	HA21_2007	+	+/FN687859	+/FN687870	–	–
	HA25_2007	+	–	+/FN687873	–	+
	HA25B_2007	+	–	+/FN687871	+	+
	HA28_2007	+	+/FN687860	–	+	–
	HA30_2007	+	+/FN687861	+/FN687872	–	–
Villa Clara	VC1_2007	–	–	–	–	+
	VC12_2007	+	–	–	–	+
	VC21_2007	+	–	–	–	+
Ciego de Ávila	CA7_2007	–	+/FN687867	–	–	+
	CA9_2007	+	+/FN687868	+/FN687876	–	+
	CA10_2007	+	–	+/FN687874	–	+
	CA18_2007	+	–	+/FN687877	–	+
	CA20_2007	+	+/FN687858	+/FN687875	–	+
	CA23_2007	+	–	–	–	+
Matanzas	MZ4_2008	+	+/FN687864	+/FN687879	–	–
	MZ5_2008	+	+/FN687865	+/FN687878	–	–
	MZ6_2008	+	+/FN687866	–	–	–
Holguín	HO1_2005	–	–	–	+	–
	HO2_2005	+	+/FN687869	–	+	–
Sancti Spiritus	SS1_2007	+	–	–	–	+
	SS2_2007	+	–	–	–	–
	SS3_2007	+	–	–	–	+
	SS4/2007	–	+/n.s	+/n.s	–	–
	SS5_2007	–	+/n.s	+/n.s	–	–
	SS6_2007	–	+/n.s	+/n.s	–	–
Cienfuegos	CF1_2007	+	–	–	+	+

n.s: Non-sequenced.

Maximum Composite Likelihood method with MEGA 4.1 software (Tamura et al., 2007). Standard error estimates were obtained by a bootstrap procedure (500 replicates).

Both TTSuV1 and TTSuV2 were found in infected pigs from Cuban swine herds. The PCR analysis showed the presence of TTVs in 52.5% (21/40); TTSuV1 in 40% (16/40); TTSuV2 in 37.5% (15/40); PCV-2 in 70% (28/40); PPV in 37.5% (15/40) and CSFV in 52.5% (21/40) of the field spleen samples assessed (Table 1). It should be noticed that 10 of the 40 (25%) pigs were infected simultaneously with TTSuV1 and TTSuV2 (Table 1).

From the 21 TTV (TTSuV1 and/or TTSuV2) positive samples, 14 (66.7%); 4 (19%) and 9 (42.9%) showed a concurrent infection with PCV-2, PPV and CSFV, respectively (Table 1). Taking the whole sample set into account, six spleen samples (15%) were positive for PCV-2, TTV and PPV at the same time whereas seven samples (17.5%) yielded positive results for PCV-2, TTV and CSFV simultaneously; and two samples (5%) were positive for PCV-2, TTV, PPV and CSFV concurrently, showing that these viruses were even found simultaneously infecting the same pig (Table 1).

This exploratory study revealed the presence of TTSuV1 and TTSuV2 for the first time in Cuban swine herds at different rates of co-infection with PCV-2, PPV and CSFV. Our results indicate that, similar to human TTV (Okamoto, 2009) and in agreement with a recent report (Huang et al., 2010; Cortey et al., 2010), multiple porcine TTSuV infections with TTSuV1 and TTSuV2 might be relatively common in pigs.

In this investigation, we observed that all these viruses (TTSuV1, TTSuV2, PCV-2, PPV and CSFV) were present at different rates (37.5–70%) in a total of 40 selected pigs. The numbers for TTSuV (52.5%) correlate well to other studies in countries such as Canada, Spain, Korea, Italy and Sweden where the rates of infected pigs ranged between 24% and 100% (Brassard et al., 2008; Martelli et al., 2006; McKeown et al., 2004; Segalés et al., 2009; Blomström et al., 2010). Likewise, the presence of multiple infections of these viruses in the same spleen sample collected from pigs with respiratory and wasting disorders is reported in this study, suggesting the possibility of synergistic effects of different viruses acting together.

The estimate of average evolutionary divergence over sequence pairs was higher within TTSuV2 (0.0994 ± 0.0158) than within TTSuV1 (0.0563 ± 0.0098). All the phylogenetic analyses based on BI, NJ, ML and MP methodologies yielded the same topology, only BI tree was shown (Fig. 1). The TTV sequences were not grouped into individual phylogenetic clades and no relationship between the sequences and the geographical region of the sample collection were found.

The phylogenetic marker used in the present work was that suggested by Segalés et al. (2009). The selected DNA fragment was not saturated, nor under selection pressure and the molecular diversity was enough to detect differences between and within genogroups (Segalés et al., 2009). Nevertheless this is a short fragment in which recombinant events have been found and part of the phylogenetic information might be lost. It is therefore possible that the lack of any observed phylo-geographical relationship could be due to the loss of phylogenetic information. Therefore, this is an issue that requires further investigation.