Veterinary Microbiology 195 (2016) 154–157

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Clinical aspects and weight gain reduction in swine infected with porcine circovirus type 2 and torque teno sus virus in Brazil

Ana Claudia de Menezes Cruz[a,*], Renato Luiz Silveira[b], Camila Freze Baez[a], Rafael Brandão Varella[a], Tatiana Xavier de Castro[a]

[a] Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense, Rua Prof. Hernani Melo, 101, CEP 24210-130 Niterói, Rio de Janeiro, Brazil
[b] Faculdade de Veterinária, Rua Vital Brazil Filho, 64, CEP 24230-340 Niterói, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 24 August 2015
Received in revised form 12 September 2016
Accepted 20 September 2016

Keywords:
PCV-2
TTSuV-1
TTSuV-2
Swine
Weight gain
Co-infection

ABSTRACT

Simultaneous Porcine circovirus type 2 (PCV-2) and Torque teno sus virus (TTSuV) infections have been reported around the world, generally linked to severe infections. In the present study, 257 swine plasma samples from 31 swine herds located in Brazil, were PCR screened for PCV-2 and TTSuV-1/2 and correlated with clinical data. PCV-2 was detected in 25%, followed by 38.1% and 42.4% of TTSuV-1 and TTSuV-2, respectively. Co-infections of two or three viruses were found in 32.3% of samples. PCV-2 was more frequently detected in the growing ($p = 0.030$) and finishing phases ($p = 0.0005$) while TTSuV-2 in the nursery ($p = 0.009$). Only TTSuV-1 was statistically associated to clinical disease (multiple signs), in combination or not with PCV-2 or TTSuV-2 ($p = 0.015$). PCV-2/TTSuV co-infections were more frequently related to weight gain reduction in comparison to mono-infections ($p = 0.049$) and no-infections ($p = 0.027$), and also in animals with ($p = 0.011$) or without ($p = 0.037$) clinical signs, being the nursery the most affected phase ($p = 0.025$). Our results uphold the pathogenic potential of TTSuV in naturally infected pigs and the clinical/economical impact of this agent, especially in co-infections. Studies addressing the physiopathological mechanisms of simultaneous infections are needed.

1. Introduction

The impairment of weaning and fattening parameters in commercial pig farms results in economic losses, higher food intake and increased feed conversion ratio (Maes et al., 2001). Among viral infections responsible for weight loss, porcine circovirus type 2 (PCV-2) plays an important role as an ubiquitous virus responsible for systemic disease (PCV-2-SD) or subclinical infection (Segalés, 2012). PCV-2 is a non-enveloped virus with a circular single-stranded DNA (ssDNA) genome that belongs to the *Circoviridae* family, with two major genotypes described (PCV-2a and PCV-2b) (Kim et al., 2001a). PCV-2 has been detected in several countries alone or in co-infections with other pathogens, resulting in an increased incidence of severe disease (Castro et al., 2012).

Another emerging agent in pig farming, Torque teno sus virus (TTSuV), is a non-enveloped virus with a circular single-stranded DNA (ssDNA) genome that belongs to the *Anelloviridae* family, and is divided into two species (TTSuV 1 and 2) and four genotypes (1a, 1b, 2a and 2b) (Martínez-Guinó et al., 2009). Although the role of TTSuV in disease remains unclear, its presence increases the severity of certain viral diseases (Ellis et al., 2008). Noteworthy, TTSuV has been found in co-infection with PCV-2 and porcine boca-like virus in samples from pigs with postweaning multi-systemic wasting syndrome (PMWS), suggesting a potential increase in morbidity (McMenamy et al., 2013).

Nevertheless, there is a scarcity of studies addressing the clinical and economical importance of co-infections, especially in regard to low feed conversion efficiency. Therefore, this study aimed the investigation of PCV-2 and TTSuV-1 and 2 presence in serum samples of domestic pigs, and its correlation with clinical signs and weight measurements.

2. Materials and methods

This study was approved by University Ethics Committee on Animal Use (CEUA number 271). We conducted a transversal study involving 257 animals from 31 swine herds from farrow to finish

* Corresponding author at: Universidade Federal Fluminense, Rua Hernani de Melo 101, Niterói, Rio de Janeiro CEP 24210-130, Brazil.
E-mail addresses: menezescruz@gmail.com, menezescruz@vm.uff.br (A.C. de Menezes Cruz).

http://dx.doi.org/10.1016/j.vetmic.2016.09.012
0378-1135/

operation located in Rio de Janeiro State, Brazil. Pigs were classified into four categories: nursery (3–10 weeks), growth (11–15 weeks), finishing (16–25 weeks), and breeding (26 weeks). PCV-2 vaccine was not included in the vaccination protocol of the herds. Clinical examination and weighing were performed before blood collection and all data were recorded for further analysis. A total of 244 animals were weighed and the measured weight was compared to the average weight of the pen. During sample collection, 150 animals showed clinical signs (enteric; respiratory; and multiple signs, defined as a combination of both), while 107 did not show any clinical symptoms. DNA was extracted from serum samples by High Pure Viral Nucleic Acid Kit® (Roche, Germany) according to manufacturer's instructions and submitted to nested polymerase chain reaction (nPCR) to detected PCV-2 and PCR to TTSuV 1 and 2, as previously described (Kim et al., 2001b; Martínez-Guinó et al., 2009). The amplicons were purified using GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare®) and subjected to direct sequencing with BigDye terminator v. 1.1 cycle sequencing kit (Applied Biosystems,CA, USA) and ABI Prism® 3730 DNA analyzer (Applied Biosystems CA). PCV-2 genotyping were performed based on pairwise comparisons of ORF2 partial sequences (Olvera et al., 2007) and phylogenetic analysis of capsid partial sequence. TTSuV-1 and 2 genotyping were performed based on phylogenetic analysis of UTR partial sequence (Martínez-Guinó et al., 2009). The Modeltest software 3.7 (Posada, 2006) was used to test for a statistically justified model of DNA substitution that best fitted our data set. A Bayesian Markov Chain Monte Carlo (MCMC) statistical framework approach was implemented in the BEAST v1.7.4 (Drummond et al., 2012). See Supplementary data. Statistical analyses were performed using SAS 6.11 (SAS Institute, Inc., Cary, North Carolina). The chi-square test or Fisher's exact test was used to examine associations between categorical variables, and the Mann-Whitney test was used for non-parametric analyses. *P* values < 0.05 were considered significant.

3. Results

Of the 257 clinical samples, the PCV-2 was detected in 64 (25%), followed by the TTSuV-1 in 98 (38.1%) and the TTSuV-2 in 109 (42.4%). Co-infections involving a combination of two or three viruses were found in 83 samples (32.3%). A total of 30 PCV-2 positive samples were sequenced and according to sequence analysis, the PCV-2 was further genotyped as PCV-2a and PCV-2b in 22/30 (73.3%) and 8/30 (26.7%) of samples, respectively. A total of 27 positive samples for TTSuV-1 were also submitted to sequence analysis and 13/27 (48%) samples were characterized as genotypes TTSuV-1a and 14/27 (52%) 1b. Twenty-nine samples for TTSuV-2 were analyzed and TTSuV-2a and 2b were detected in 23/29 (79%) and 6/29 (21%) of samples, respectively. All the nucleotide sequences generated in this study were deposited in the GenBank with the following accession numbers: KX833728-KX833813.

The Table 1 summarizes the occurrence of the investigated viruses according to production phase. The PCV-2 was more frequent in the growing ($p = 0.030$) and finishing ($p = 0.0005$) while TTSuV-2 was frequently found in the nursery ($p = 0.009$). The TTSuV-1 was not statistically predominant in any production stage.

Of the 150 animals presenting clinical manifestations, 96 (64%) had at least one out of the three viral infections studied [positive predictive value (PPV) = 55.5% (95% CI: 52.1–64.5) and negative predictive value (NPV) = 35.7% (95% CI: 25.5–46.9); ($p = 0.180$)]. Animals presenting clinical signs were more frequently co-infected than those mono-infected were, although such difference did not reach statistical significance ($p = 0.064$). Among the clinical findings, the occurrence of multiple signs was statistically associated with TTSuV-1 (Table 1). The PCV-2 and TTSuV-2 were not significantly associated to any clinical manifestations either in combination or in single infections.

Of the 244 animals whose weight was measured, 182 (74.6%) had weight gain reduction (median reduction: $18\% \pm 11.82$) when compared to average weight of the pen and, of these, 132 (72.5%) were infected with one or more viruses ($p = 0.019$). Interestingly, there was no difference in weight gain reduction in mono-infections (median reduction: $11.1\% \pm 10.7$) in comparison to no viral detection (median reduction: 10.0 ± 13.9) ($p = 0.642$). However, weight gain reduction was significant higher in co-infection (median reduction: $19.1\% \pm 14.4$) in comparison to no viral detection ($p = 0.027$) and mono-infections ($p = 0.049$) (Fig. 1a). Co-infected animals had over than 40% weight gain reduction in comparison to those mono-infected and without viral detection, regardless the combinations of viruses involved ($p = 0.608$) (Fig. 1b).

When weight gain reduction was compared to clinical manifestations, it was possible to observe that co-infections influenced the weight of animals with ($p = 0.011$) or without ($p = 0.037$) clinical signs (Fig. 2). Concerning to the production stage, the presence of co-infections was related to a significant weight reduction in the nursery ($p = 0.025$) and a strong tendency in the growing phase ($p = 0.064$), representing a general weight reduction over than 48% in comparison to mono-infection and no viral detections.

4. Discussion

PCV-2 and TTSuV have been studied in different regions of Brazil and are considered important agents related to economic losses in pig farming (Castro et al., 2007; Pinto et al., 2011).

In this study, PCV-2 was detected in 25% of samples tested at different stages of production, similarly to another report

Table 1
Viral detection according to production phases and clinical signs (N = 257).

Characteristic	PCV-2 (N = 64)	p value	TTSuV-1 (N = 98)	p value	TTSuV-2 (N = 109)	p value
Production phase	n (%)		n (%)		n (%)	
Nursery (N = 166)	27 (16.3)	NS	70 (42.2)	NS	**78 (47)**	0.009
Growing (N = 65)	**23 (35.4)**	0.030	21 (32.3)	NS	19 (29.2)	NS
Finishing (N = 21)	**11 (52.4)**	0.0005	5 (23.8)	NS	12 (57.1)	NS
Breeding (N = 5)	3 (60)	NS	2 (40)	NS	0	NS
Clinical signs	n (%)		n (%)		n (%)	
Enteric (N = 31)	8 (25.8)	NS	8 (25.8)	NS	7 (22.6)	NS
Respiratory (N = 95)	20 (21)	NS	30 (31.6)	NS	40 (42.1)	NS
Multiple (N = 24)	8 (33.3)	NS	**17 (70.8)**	0.015	12 (50)	NS
No signs (N = 107)	28 (26.1)	NS	43 (40.2)	NS	50 (46.7)	NS

PCV-2: porcine circovirus type 2; TTSuV 1: torque teno sus virus type 1; TTSuV 2: torque teno sus virus type 2. Bold letters: p-value <0.05; NS: non-significant.