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Brief Original Article

Molecular survey of Cytomegalovirus shedding profile in commercial pig herds in Brazil

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Abstract

Introduction: Porcine cytomegalovirus (PCMV) causes rhinitis in both young and older pigs. The present study describes the detection and characterization of shedding profiles of PCMV in nine farrow-to-finish Brazilian swine herds.

Methodology: Tonsil swabs from sows, nursery and grow-finish pigs of nine farrow-to-finish commercial herds (n = 756) were evaluated for the presence of PCMV by PCR.

Results: The virus was detected in all herds. Positive samples were concentrated in piglets of ages varying from 40 to 60 days (nursery phase), while none of the sows were positive for PCMV detection.

Conclusions: These findings corroborate the literature regarding PCMV worldwide distribution, and introduce the first report of PCMV shedding profile in Brazilian pig farms.

Key words: porcine cytomegalovirus; swine; PCR; shedding profile.

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Introduction

Porcine Cytomegalovirus (PCMV) belongs to the β-*herpesvirinae* family and, despite being species-specific, it shares homology with cytomegaloviruses of human and other animals [1]. PCMV infection is usually subclinical in older pigs, but similar to the human cytomegalovirus, it can cross the placenta and often produces mummification, abortion, low viability of piglets at birth and consequential pre-weaning mortality [2]. In susceptible herds, the virus also causes runting, rhinitis, pneumonia [3-5] and neurological disorders [6].

PCMV is a widespread virus with herd prevalence greater than 90% in Europe, North America and Japan [1]. Virus transmission occurs horizontally via the oronasal route, but congenital transmission is also well documented [2]. Infection most commonly occurs perinatally or early postnatally in commercial herds, and the majority of pigs shed PMCV in nasal secretion between three and eight weeks of age [1].

To enhance the knowledge of this virus in Brazilian herds, the present study describes the detection and characterization of shedding profiles of PCMV in nine farrow-to-finish Brazilian swine herds.

Methodology

Tonsil swabs were collected from commercial pig farms (A to I) in São Paulo State, southeast of Brazil (Figure 1). Twelve tonsil swabs from sows, piglets and pigs with 40, 60, 90, 110 and 140 days of age were collected, in a total of 84 swabs in each herd. The animals were randomly selected for tonsil swab collection. After collection, all 756 swabs were soaked in buffered saline (0.9%) and frozen at -20°C until their processing. The samples were divided according to the animal age and the corresponding production phase (Farrowing – sows and 20 days of age piglets; Nursery - 20 to 40 days; Grown-finish – 90 to 140 days of age).

Purified DNA was recovered from the clinical samples using Boom *et al.* [7] protocol. PCMV molecular detection was performed using Hamel *et al.* [8] primers. PCR was performed for 35 cycles consisting of denaturation at 95°C for 1 minute, annealing at 60°C for 1.5 minutes and extension at 72°C for 1 minute. The amplified products were subjected to

electrophoresis in 1.5% agarose gel (LGC Biotecnologia, São Paulo, Brazil), stained with BlueGreen (LGC Biotecnologia, São Paulo, Brazil). The 100 bp DNA ladder (LGC Biotecnologia, São Paulo, Brazil) was used for molecular weight determination.

Positive controls were obtained from the nasal mucosa of two piglets diagnosed with inclusion body rhinitis, by means of histopathological examination and electron microscopy (data not shown). Chi-Squared test was performed to verify the association between production phases and virus shedding, at a significance level of 1%, using Minitab 15 (Minitab Inc, Pennsylvania, USA).

Figure 1 was built using the software – ArcGIS Desktop Help 10.3 Geostatistical Analyst. - Environmental Systems Research Institute (ESRI California, USA), (2015). The data was extracted from -IBGE, digital municipal mesh of Brazil. São Paulo: IBGE, 2014. (http://www.ibge.gov.br).

Results

From the 756 investigated samples, 198 (26.2%) were positive for PCMV. The virus was detected in all nine herds (Table 1), concentrated on pigs between 40 and 60 days of age (Nursery phase), which represent 71.2% of all positive cases (Figure 2). With exception in sows, PCMV was detected in all stages of production. Examining herds individually, it was possible to observe that only at farms A and H, PCMV was detected in animals from 20 to 140 days of age. Segregating samples according to production phases (farrow, nursery and grown-finish), PCMV was detected at the following frequencies: 15/756 (1.98%), 141/756 (18.65%) and 42/756 (5.57%), respectively (Figure 3). The association between the nursery phase and PCMV excretion was statistically significant ($p \leq 0.01$).

Figure 1. Map representing the distribution of the nine evaluated herds (A to I) in São Paulo State, Brazil.

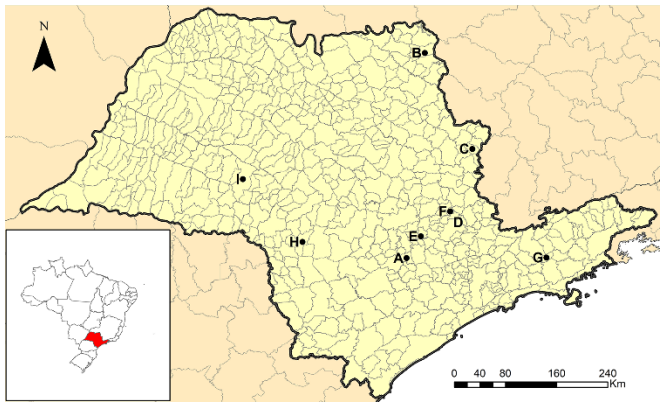

Figure 2. Number of PCMV-positive samples at each age examined, in all nine Brazilian herds.

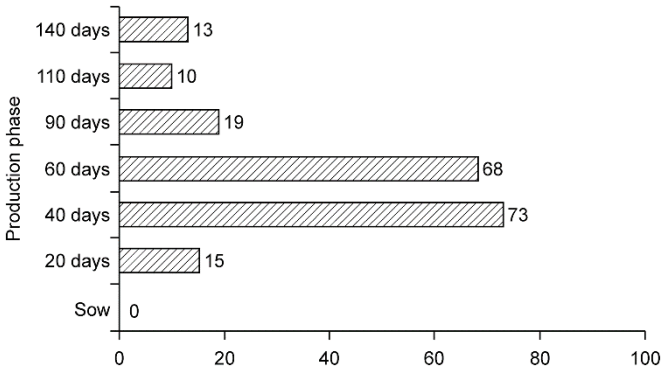

Figure 3. Frequency of PCMV-positive samples at each phase examined, in all nine Brazilian herds.

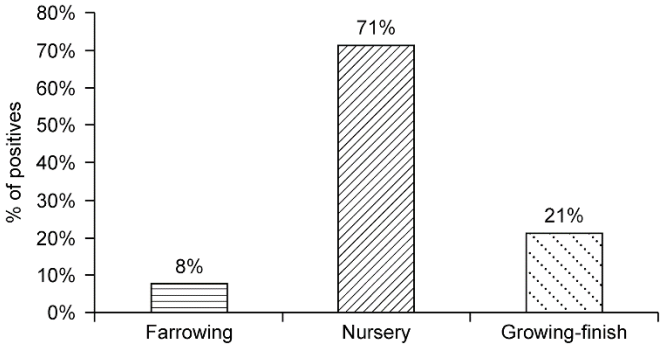

Table 1. Number of PCMV-positive animals in each swine herd discriminating the different ages of examined pigs.

Herd	N (%) positive animals/ herd	Farrowing		Nursery		Grown-finish		
		Sow	20 days	40 days	60 days	90 days	110 days	140 days
A	29 (34.5)	0/12	2/12	11/12	9/12	4/12	1/12	2/12
B	16 (19.0)	0/12	4/12	10/12	2/12	0/12	0/12	0/12
C	24 (28.6)	0/12	5/12	10/12	6/12	3/12	0/12	0/12
D	15 (17.8)	0/12	1/12	2/12	7/12	2/12	0/12	3/12
E	20 (23.8)	0/12	1/12	4/12	11/12	1/12	0/12	3/12
F	19 (22.6)	0/12	0/12	7/12	7/12	2/12	3/12	0/12
G	22 (26.2)	0/12	0/12	8/12	7/12	2/12	4/12	1/12
H	29 (22.6)	0/12	2/12	11/12	9/12	4/12	1/12	2/12
I	24 (28.6)	0/12	0/12	10/12	10/12	1/12	1/12	2/12
Total	**198 (26.2)**	**0/108**	**15/108**	**73/108**	**68/108**	**19/108**	**10/108**	**13/108**

Discussion

Our results demonstrate that PCMV infection is widely spread among the studied farms. These corroborate previous reports that PCMV is a commonly virus whose antibodies, as well as DNA, have been found in high percentages in swine herds worldwide [9]. In the United Kingdom, 50% of the herds were reported as infected, while the seroprevalence in Japan and the Netherlands was extremely high, with more than 99% and 93% of tested pigs positive, respectively. Prevalence of PCMV among Canadian pigs was reported to be 59% by PCR [8,10-13].

None of the sows examined in this study were positive for PCMV, which can be explained by the viral latency within lung macrophages, without PCMV necessarily being eliminated [1]. Despite the difficulty of the *in vitro* cultivation of the virus, PCMV has been shown to infect porcine primary pulmonary macrophages and epithelial-like and fibroblast-like cells. The ability to infect lung macrophages raises some concern that PCMV may modify host defense mechanisms and alter the pathogenic consequences in the host [14].

The association between the PCMV shedding and the nursery phase is consistent with Yoon and Edington [1] review that stated that the virus excretion is concentrated between three and eight weeks (21 and 56 days) of age, although in some cases it may occur after ten weeks with high morbidity. The greatest PCMV excretion at the nursery phase has also been related to post-weaning stress, the practice of mixing together several litters, environmental changes and the progressive decline of maternal immunity [1]. This reinforces the importance of good management systems in pig production, especially in these critical stages of breeding.

Conclusions

These findings corroborate the literature regarding PCMV widely distribution, and introduces the first report of PCMV shedding profiles in Brazilian swine herds demonstrating the need of diagnostic tests and monitoring for this infection.

Acknowledgements

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