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Phylogenetic characterization of PCV2 in Malaysia

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Introduction

PCV2 is a virus of huge economic significance worldwide. It is identified as the causative agent of PCV-associated diseases (PCVAD) or also referred to as postweaning multisystemic wasting syndrome (PMWS). Globally, the virus has been reported in Canada, US, several European countries and in Asia. In Malaysia PCV2 was first identified by the Veterinary Research Institute in 2004 by RFLP methods followed by the first case study of PCVAD in 2007 based on clinical features, histopathology findings and PCR screenings [1]. It is hypothesized that PCV2 is widely distributed in the country and that PCV2b is the more dominant subtype found in Malaysia. Therefore, the objectives of this study would be to detect PCV2 from selected pig farms and to genetically characterize the PCV2 found in this study. Understanding the presence of the virus may contribute to better vaccination programs against the virus and disease. At the time the samples were collected, PCV2 vaccines were not available in Malaysia. This study will represent the complete phylogenetic characterization of PCV2 reported for the first time in Malaysia from samples collected in 2007.

Materials and Methods

Sampling and Screening of PCV1 and PCV2 by PCR

Organ samples were collected from 42 pig farms in Malaysia from animals displaying classic PCVAD clinical signs. Nucleic acid extraction was done by using Trizol LS reagent according to the manufacturer's protocol. The nested multiplex PCR was carried out as described previously by Kim et al. 2003[2].

PCR amplification of the complete genome of PCV2

Amplification of the whole genome of PCV2 was carried out by amplifying two overlapping fragments of the entire PCV2 genome according to a previously described method [3, 4].

Nucleotide sequencing, sequencing analysis and construction of phylogenetic tree

Sequencing of the complete genome of PCV2 was done in a commercial sequencing facility. Sequence editing and assembly were done by using ClustalW and BioEdit. The phylogenetic tree was constructed through the distance-based neighbor joining method and generated by using Mega 5 (Biodesign Institute, Tempe, Arizona).

Results

This study showed that 37 out of 42 farms sampled were positive for PCV2 by PCR screening. Thirteen whole genomes of PCV2 isolates from pigs with typical PCVAD symptoms were successfully sequenced. These isolates shared 98.3-99.2% similarities with sequences of isolates from the Netherlands. All thirteen isolates fell into the same clade as PCV2b isolates from other countries. Amino acid sequence analysis of the putative capsid protein (ORF2) of the PCV2 revealed that there are three clusters found in Malaysia, namely cluster 1C and 1A/1B. Of interest, three of the isolates had a proline substitution for arginine or isoleucine encoded at nt. position 88-89. Eight of the isolates had mutations at the C terminus of the putative capsid protein suggestive of higher pathogenicity which may account for the high reports of PCVAD clinical symptoms in 2007.

Conclusions and Discussion

Findings from this study confirm that PCV2 is widely distributed in Malaysia and PCV2b is the more dominant subtype found in this country. Comparable to the PCV2 findings in Malaysia, neighbouring countries such as Thailand and Indonesia have reported the presence of PCV2b cluster 1C and cluster 1A/B respectively suggesting that similar PCV2b clusters as reported in this study are circulating in these few Asian countries. This suggests that the swine in this region could be harbouring the virus from the same source and that there may be a link between movements of animals by import of breeders into the country being the route of entry of the virus.

It is not possible to eradicate the virus from commercial pigs; however the swine industry in Malaysia can be safeguarded by control measures implemented throughout the country. These measures should include improved biosecurity, disease surveillance; vaccination as well as enforcement of regulations formulated to control and prevent the spread of the disease.

References

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