FACULTY OF VETERINARY MEDICINE
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INVITATION

PUBLIC DEFENCE OF THE DOCTORAL THESIS

Better insights in porcine circovirus type 2 (PCV2) epidemiology and cellular pathogenesis

Ruifang Wei

GHENT UNIVERSITY

Promoter

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Sokoine University of Agriculture

Dr. Tom Meyns
National Veterinary Manager, Benelux at Zoetis

Curriculum Vitae

Ruifang Wei was born in Henan Province, China, on July 14th, 1990. In September 2011, she was enrolled as a master student in Northwest Agriculture and Forestry University, where she studied the interaction between porcine reproductive and respiratory syndrome virus (PRRSV) and its putative receptor. She received a travel grant to attend the 2013 International PRRS Symposium, Beijing, China.

In September 2014, she started her PhD research in the Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, financed by the Chinese Scholarship Council. Her research focused on the epidemiology and pathogenesis of porcine circovirus type 2 (PCV2). In 2015, she received a European PCV2 Research Award from Boehringer Ingelheim, and finalized the project with excellence in the next year.

During her PhD, she successfully completed the full curriculum of the Doctoral Training Programme, organized by the Ghent University Doctoral School. She is author/co-author of seven publications in national and international journals. She has given eight oral/poster presentations in national and international scientific congresses/symposia.

When and where

The public defence will take place on

Thursday 21th March at 17.30
Auditorium Hoogbouw (Entrance 24)
Faculty of Veterinary Medicine, UGent
Salisburylaan 133, 9820 Merelbeke

A reception with drinks and traditional Chinese food will follow in the Museum of Anatomy ☺

Registration

If you plan to attend the reception, please confirm your presence before 18 March by e-mail to ruifang.wei@ugent.be

Summary of the Thesis

Porcine circovirus type 2 (PCV2) is a ubiquitous virus that infects young pigs after weaning. The majority of these infections occurs sub-clinically. Under certain conditions, PCV2 infection is associated with postweaning multisystemic wasting syndrome (PMWS), a multifactorial disease characterized by lymphocyte depletion and monocyte infiltration in lymphoid tissues. Nevertheless, PCV2 pathogenesis is largely unknown. The aims of this study were to study PCV2 genotypic evolution in Belgium, to investigate PCV2 entry and viral outcome in monocytes and T-lymphoblasts, and to identify critical factors in the switch from PCV2 sub-clinical infection to clinical infection.

In the first study, we demonstrated that at least three genotypes (PCV2a, PCV2b and PCV2d) circulated in Belgium from 2009 till 2018, and that PCV2 evolved from PCV2a to PCV2b and from PCV2d-1 to PCV2d-2. Sequence comparison among the 43 PCV2 isolates showed that they had 89.7-100% nucleotide-sequence and 88.5-100% amino-acid-sequence identities. Three-dimensional analysis of genotype-specific amino acids revealed that most of the mutations were on the outside of the cap protein and a few conserved ones on the inner side. Mutations towards more basic amino acids were found on the upper and tail parts of two connecting capsid proteins which form one big contact region, most probably involved in receptor binding. The lower part was relatively conserved. These polarity changes together with the formation of an extruding part might drive the virus to a more efficient glycosaminoglycans receptor binding.

In the second study, we demonstrated that, upon the addition of PCV2 particles to monocytes, PCV2 entered monocytes via clathrin-mediated endocytosis, became partially disintegrated in the endosome-lysosome system, and finally remnants ended up in lysosomes. During the endosomal trafficking, the PCV2 genome escaped from the endosomal system and was present in monocytes in its complete form. Monocytes from purebred Piétrain and hybrids showed a higher level of PCV2 uptake and disintegration, compared to those from Landrace and Large White. This may partly explain the breed difference in PCV2 susceptibility in the field.

In the third study, we demonstrated that, *in vitro* generated porcine T-lymphoblasts supported the replication of PCV2. Virus binding was partly mediated by chondroitin sulfate, followed by an entry via clathrin-mediated endocytosis. The incoming virus required a cleavage by serine proteinases in an acid environment for virion disassembly. Furthermore, PCV2 abortion strain 1121 but not PMWS strain Stoon1010 was able to exploit macropinocytosis (non-specific, cell-drinking) as an additional entry pathway. Three-dimensional analysis of the cap structure predicted a better cap-nucleic acid affinity of Stoon1010 than 1121. We hypothesize an evolution from 1121 to Stoon1010, as revealed by (i) an enhanced virion binding to cells, (ii) a more specific receptor-mediated entry into cells, and (iii) an increased affinity of viral capsids to viral nucleic acids of Stoon1010 compared to 1121.

Lastly, we demonstrated on a transcriptomic level that, subclinical and clinical PCV2 infection represent two diametrically opposed transcriptomic recalibrations, with disabled immune system networks in the former, and an activated immune response in the latter. Hallmark gene set analysis revealed that IL-2 mediated immune activation of lymphocytes was a hallmark of a fulminant viremia. IL-2 treatment of T-lymphoblasts in vitro enhanced cell survival and PCV2 replication in the cells. Furthermore, functional genomics analysis of hallmark gene sets demonstrated that STAT3 was a druggable PCV2 host factor. Cpd188, a STAT3-specific inhibitor, impaired PCV2 infection in lymphoblasts. We concluded that the transcriptional input initially set by subclinical PCV2 was overridden by an extensive inflammatory response in a PMWS condition after superinfection, thus triggering PCV2 viremia and PCV2 disease.

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