Molecular processes of reconstructed porcine circovirus type 2 genotype group infections in weaners

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Key words
PCV2 genotype group members, weaner, experimental infections, PCV2 DNA from paraffin embedded tissue

Aim of the study
We tested rigorously whether porcine circovirus type 2 (PCV2) infections alone might be sufficient to induce postweaning multisystemic wasting syndrome (PMWS) in weaners. In the field, PMWS is well described with presence of PCV2 and other infectious pathogens. However, these cofactors superimpose their effects on PCV2 pathogenesis. Hence, it is unclear whether a cofactor is needed to initiate PMWS or multiple, more virulent PCV2 suffice. We developed a weaner infection model to delineate molecularly PCV2 pathogenesis.

Material and methods
Whole PCV2 genomes were reconstructed from paraffin embedded pig tissue with aid of a proof-reading polymerase. These were protected with *in vivo* transfection reagent (Brunschwig) and injected into weaners neck. We administrated immunosuppressive cyclosporine A (CysA) 30mg/kg and day as cofactor. 20 weaners were divided into 6 groups with 2 weaners' in the control-group and 2 weaners' receiving CysA (control-group+CysA), remaining weaners' were distributed within 4 groups injected with respective genotype group member cocktail: PCV2a-group, PCV2b-group, PCV2a/b-group and PCV2a/b+CysA-group. We measured PCV2 DNA load by quantitative PCR, IgM' and IgG' with aid of respective ELISAs', PCV2 antigen or PCV2 DNA was visualized by immunohistochemistry (IHC) or by fluorescence in situ hybridization (FISH). B- and T-cells from blood and organs were distinguished by flow cytometry. Two pilot experiments added to study *in vivo* transfections and CysA-effects.

Results and significance
PCV2 *in vitro* production is challenging and yet these virulent. Hence, we constructed 24 whole recombinant PCV2 genotype group members including 8 new members (2 whole PCV2a and 6 whole PCV2b). Due to a novel *in vivo* transfection protocol we needed about 25 fold less DNA for infections than suggested. Newly infected pigs were viremic 13, 16 or 27 days post infection (dpi) for PCV2a/b-PCV2b-, PCV2a/b+CysA- or PCV2a-group, respectively. Additionally, we can show that injected PCV2 underwent evolution as we had speculated previously. Only PCV2a-group showed transient viremia. This correlated with PCV2 specific IgM expression. In other infected pigs we observed a spike to moderate levels of PCV2 specific IgG' at day 41 dpi. PCV2a/b+CysA-group pigs contained in general 10 times more PCV2 with a peak of several 100 millions per ml blood. Nevertheless, significant PCV2 antigen was only in PCV2a/b+CysA-group pigs observed, indicating CysA carried PCV2 persistency in secondary lymph organs. While PCV2a/b+CysA-group pigs harbored over 95% replicative PCV2 isoform**s** controls contained less than 10%. Thus, cofactors influencing the immune system seem to be necessary to facilitate PCV2 persistency and replication. Concomitant this might indicate cytotoxic T-cells responses are functional hampered.

Publications, posters and presentations
2 internal presentations, 1 oral presentation at the IPVS (2012) in South Korea, page 101: "Characterization of reconstructed porcine circovirus type 2 genotypes infections in weaners", 8 newly discovered PCV2 whole genome sequences deposited in the NCBI database, 2 manuscripts in preparation: i) PCV2 evolution in pigs and ii) PCV2 interactions with the host immune system.

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