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Comparative analysis of type 1 and type 2 porcine reproductive and respiratory syndrome viruses *in vitro*: macrophage tropism and genetic determinants of the different replication characteristics

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Key words
Porcine reproductive and respiratory syndrome virus, PRRSV, *Arteriviridae*, *Nidovirales*, genotype, pig, macrophage, tropism, virulence, replication, pathogenesis, diagnostics

Aim of the study
Porcine reproductive and respiratory syndrome virus (PRRSV) is endemic in Europe, while Switzerland is currently free. However a short outbreak in Switzerland in 2012 due to accidental introduction of the virus from Germany through import of contaminated semen from an acutely infected boar emphasized the continuous threat for our country (Nathues C. *et al.,* 2016, Transbound. Emerg Dis. 63:e251-61). In order to improve preparedness and to establish a centre of expertise for PRRS at IVI, this project was aimed at implementing the necessary technical and scientific environment to study the virus and control the disease. The objectives were to: (i) implement reverse genetics for PRRSV genotype 1 and 2 prototype strains to study viral-host interactions; (ii) identify macrophage-specific cellular factors that support efficient replication of natural PRRSV isolates and (iii) generate PRRSV-permissive permanent porcine cell lines for propagation of PRRSV *in vitro*.

Material and methods
The PRRSV isolate IVI-1173 was recovered in porcine macrophages from serum of an infected pig that was diagnosed PRRSV-positive during the short Swiss outbreak in 2012. A second PRRSV, the highly pathogenic RVB-581, was isolated at the Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany and obtained from Martin Beer. RT-PCR, cDNA cloning and nucleotide sequencing were applied to analyze these viruses genetically and to rescue functional cDNA clones for reverse genetics studies. The antigenic and replicative properties of these two viruses were characterized extensively in primary macrophages and other permissive cells. Genetic and biochemical approaches were used to identify critical cellular factors for PRRSV replication.

Results and significance
The complete genome sequences of the PRRSV isolates IVI-1173 and RVB-581 were determined and deposited to GenBank (accession numbers KX622783 and KX650082, respectively). Phylogenetic analyses revealed that IVI-1173 is a genotype 1 subtype 1 PRRSV with only 89% nucleotide identity with the closest related PRRSV genome sequence known and 61% identity with the genotype 2 isolate RVB-581. Functional cDNA clones were established successfully for the two viruses (pIVI1173 and pRVB581), providing the IVI with a powerful toolbox to study the PRRSV biology and PRRS pathogenesis using reverse genetics. A recombinant genome carrying the EGFP marker gene was constructed for screening purposes to select for permissive cells. Such chimeric genomes can also be applied as internal standards for diagnostic quantitative RT-PCR. Interestingly, the IVI-1173 isolate possesses atypical genomic and antigenic features. In particular, a monoclonal antibody (mAb) used routinely for immunostaining of the PRRSV nucleocapsid protein N in virus isolation protocols did not detect IVI-1173-infected cells. Using the functional pIVI1173 and pRVB581 clones, we demonstrated the critical role of amino acid 90 of the N protein for reactivity with this mAb. We then screened all PRRSV N sequences from GenBank and found an unexpected high prevalence of amino acid substitutions at this position of N predicted to abolish the reactivity with the mAb. These clustered essentially in the Russian subtype 1 and

in the subtype 2 strains. Therefore, these findings are of outmost importance for the diagnosis and surveillance of PRRS (Rappe *et al.*, submitted for publication).
A characteristic of the virus is its restricted cell tropism, limiting replication to macrophages and MARC-145 cells. However, plasmacytoid dendritic cells can sense infected macrophages resulting in high type I interferon production in absence of replication in these cells (García-Nicolás *et al.*, 2016). Numerous attempts to select for PRRSV-permissive porcine macrophages immortalized by lentiviral gene transduction failed. Immortalized clones from bone marrow lost the capacity to support virus replication. Finally, using an antiviral compound, we demonstrated the requirement of intracellular double membrane vesicles for PRRSV replication (Rappe *et al.*, publications in preparation).
In conclusion, together with three concurrent EU-funded projects (PoRRSCon, ANIHWA KILLeuPRRSV and SAPHIR), the present study paved the way for PRRSV research at IVI which is now internationally recognized in the field. Critical issues for PRRSV isolation were identified, which helped improving the standard operating procedures of the diagnostic laboratory. In addition, selected results from the present project were exploited in a grant application submitted to the Swiss National Science Foundation for future more fundamental research in the field.

Publications, posters and presentations

Rappe, J.C.F.; Stalder, H.; Thiel, V.; Ruggli, N. (2015) Inhibition of Porcine Reproductive and Respiratory Syndrome Virus and Equine Torovirus Replication by Targeting the Formation of Double-Membrane Vesicles. 25th Annual Meeting of the Society for Virology (GfV), 18-21 March 2015, Bochum, Germany, *poster presentation (P84).*

Rappe, J.C.F.; Thiel, V.; Ruggli, N. (2015) Inhibition of PRRSV replication by targeting the formation of double-membrane vesicles. International PRRS Congress, 3-5 June 2015, Bochum, Germany, *oral presentation (O-08).*

Rappe, J.C.F.; Ruggli, N. (2016). Heterogeneous antigenic properties of the porcine reproductive and respiratory syndrome virus (PRRSV) nucleocapsid among genotype 1 strains. 74th Annual Meeting of the Swiss Society for Microbiology, 13–15 June 2016, Bern, Switzerland, *oral presentation (O-163).*

Rappe, J.C.F.; Renzullo, S., Hofmann, M.A.; Ruggli,N. (2016) Porcine reproductive and respiratory syndrome virus isolate IVI-1173, complete genome. GenBank accession number KX622783.

Rappe, J.C.F.; Wernike, K.; Beer, M.; and Ruggli, N. (2016) Porcine reproductive and respiratory syndrome virus isolate RVB-581, complete genome. GenBank accession number KX650082.

García-Nicolás, O.; Auray, G.; Sautter, C.A.; Rappe, J.C.F.; McCullough, K.C.; Ruggli, N.; Summerfield, A. (2016) Sensing of porcine reproductive and respiratory syndrome virus-Infected macrophages by plasmacytoid dendritic cells. Frontiers in Microbiology 7:771.

Rappe, J.C.F.; García-Nicolás, O.; Flückiger, F.; Thür, B.; Hofmann, M.A.; Summerfield, A.; Ruggli, N. (2016) Heterogeneous antigenic properties of the porcine reproductive and respiratory syndrome virus nucleocapsid. Vet. Res. 47(1):117

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Rappe, J.C.F.; V'kovski, Ph.; Thiel, V.; Ruggli, N. Mapping of adaptive mutations conferring resistance to a chemical compound targeting the double membrane vesicles formation during PRRSV replication, *in preparation.*

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