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Establishing preparedness for PEDV outbreaks

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Key words
Porcine epidemic diarrhea virus (PEDV), diagnostic qRT-PCR, outbreak preparedness, virus pathogenicity

Aim of the study
Porcine epidemic diarrhea (PED) is a devastating enteric disease of pigs that poses severe economical and animal health threats. Although PED outbreaks were not observed in Switzerland since more than 20 years, recent increase in outbreaks in China and transmission of PEDV to North-America exemplified that PEDV may become a problem in Europe. In order to raise preparedness for future PEDV outbreaks this project aims to:

(i) to revise and update diagnostic tools in order to detect all current and past PEDV strains with high specificity and sensitivity,

(ii) to establish suitable experimental systems to evaluate pathogenicity of currently circulating PEDV strains,

to clarify if the developed diagnostic tests will be able to detect the currently circulating PEDV strains from field samples

Material and methods
To revise and update diagnostic tools with the focus on designing a qRT-PCR to detect all known PEDV strains all available full length genomes and selected genes of PEDV were retrieved from Genbank. Referring to this data we performed genome wide alignments to detect conserved regions for the selection of appropriate primers and probes. The resulting PCR primers and probes were thoroughly evaluated. To establish a suitable experimental system to evaluate PEDV pathogenicity we established a reverse genetic system by cloning a full-length cDNA of a high-pathogenic PEDV strain (Minnesota; MN). Subsequent rescue of recombinant PEDV-MN was achieved by in vitro transcription of an infectious PEDV-MN RNA and electroporation into BHK-cells. The obtained supernatant containing up to 10^5 infectious PEDV-MN particles was used to infect 7 day old piglets and pathogenicity was assessed. Beside PEDV-MN, we used also used recombinant PEDV-MN viruses containing the spike gene of PEDV-CV777 (attenuated laboratory stain) and PEDV-MN with a disrupted ORF3 gene (according to PEDV-CV777).

Results and significance
Based on a comprehensive phylogenetic analysis using >1000 PEDV sequences (GenBank) we could gain insight into the worldwide distribution of several genetic PEDV variants, providing an estimate of strains that might reach Europe within a possible future outbreak. Surprisingly, this analysis also revealed a likely origin of highly pathogenic US-PEDV strains is Asia, but not China. We could also identify highly conserved regions that were used to set qRT-PCR primers and probes. Subsequently, a qRT-PCR protocol was established and thoroughly evaluated. Data were communicated to Project Partner Dr. Alex Räber (Thermo-Fisher) and to Dr. Claudia Bachofen (Diagnostics, Virology Zürich).
Based on the genome sequence of PEDV Minnesota (PEDV-MN) at GenBank we have in collaboration with our Partner Prof. Gergely Tekes, Justus-Liebig-University, Giessen, cloned the entire PEDV-MN genome in vaccinia virus and have established a reverse genetic system for PEDV. The recombinant PEDV-MN displayed a

high-pathogenic phenotype in 7 day old piglets. Moreover, by replacing the spike gene of PEDV-MN with the spike gene form the laboratory strain PEDV-CV777 we could show that the recombinant chimeric virus PEDV-MN-S_{CV777} is attenuated, demonstrating the the spike gene is a major determinant of PEDV pathogenesis. In contrast, a chimeric virus containing the (interupted) ORF3 gene from PEDV-CV777 remained high-pathogenic, demonstrating that the PEDV ORF3 gene has not a major impact on PEDV pathogenicity.
The experimental infection was highly informative concerning the phenotype of high-pathogenic PEDV strains, since we can expect that such infection, should they appear in Switzerland, would not happen unrecognized. The obtained and stored samples from the experimental infection will serve as reference material in case PEDV infection may be observed in the future in Switzerland.

Publications, posters and presentations (Formatvorlage Überschrift 2)
We are currently preparing two manuscripts describing (i) the reverse genetic system and the in vivo experiments, and (ii) the qRT-PCR and the phylogenetic analysis.

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