Virulence plasmid characterization of porcine Clostridium perfringens type C

Horst Posthaus1, Vincent Perreten2, Gudrun Overesch2, Werner Zimmermann3
1Institute of Animal Pathology, 2Institute of Veterinary Bacteriology, 3Porcine Clinic, Vetsuisse Faculty, University of Berne, Switzerland

Key words
Clostridium perfringens, antibiotic resistance, genetic relatedness, cpb plasmid

Aim of the study
The aim of our study was to determine the genetic relatedness and antibiotic resistance profiles of porcine Clostridium perfringens type C isolates, which cause fatal necrotizing enteritis in newborn pigs. Additionally, we aimed to sequence the beta-toxin gene (cpb) carrying plasmid of these isolates to determine potential plasmid encoded co-factors for the disease or co-selection of antibiotic resistance.

Material and methods
A total of 44 C. perfringens type C isolates were investigated using Pulsed-Field-Gel-Electrophoresis and plasmid profiling. Antibiotic resistance profiles were determined by MIC and detection of antibiotic resistance genes. Plasmid DNA preparations were sequenced using both Illumina and Ion Torrent technologies.

Results and significance
We identified 12 different PFGE types in Swiss isolates, and the Belgian isolates clustered in three additional PFGE types. On several farms, isolates obtained from the same outbreak showed different PFGE types. All isolates were resistant to tetracycline. Additionally, resistance against cefotetan (n=1), clindamycin (n=7), metronidazole (n=6) and penicillin (n=8) were detected. Several isolates showed decreased susceptibility to piperacillin-tazobactam, ampicillin, chloramphenicol, piperacillin and meropenem. Our study showed that NE in Switzerland is caused by more than one clonal lineage of C. perfringens type C. Resistance to β-lactam antibiotics as well as clindamycin indicates that resistance to clinically important antibiotics has been selected in porcine C. perfringens isolates. Plasmid DNA from two isolates of interest were selected for sequence analysis. Sequencing generated several contigs which contained the beta-toxin gene, the plasmid replication region as well as the conjugative regions. Multiple long PCRs have been performed to close sequence gaps between the different contigs. PCR are still ongoing to close gaps between the different contigs and confirm the final assembly of the entire beta-toxin plasmid. Because, resistance to β-lactam antibiotics in Clostridia is poorly understood, we will additionally use and extend these sequence analyses to investigate the basis of this resistance in a follow up project at the Institute of Veterinary Bacteriology.

Publications, posters and presentations
A. Candi, V. Perreten, F. Van Immerseel, H. Posthaus. Clonal relationship of porcine C. perfringens type C isolates. FEBS Spetses Summer School 2012: Pathogen-host-interactions of major animal infectious diseases and zoonoses, Greece, 9-15 September 2012

A. Candi: Clonal relationship and antimicrobial resistance in Swiss porcine C. perfringens type C isolates. 8th International Conference on the Molecular Biology and Pathogenesis of the Clostridia, Palm Cove, Australia, Oct. 2013

A. Candi, V. Perreten, F. Van Immerseel, H. Posthaus. Clonal elationship and Antimicrobial Susceptibility of Porcine Clostridium perfringens type C Isolates from Switzerland. In preparation, submission to International Journal of Microbiology (open access) in October

A. Candi. Clonal relationship, antimicrobial susceptibility, and virulence plasmid characterization of porcine Clostridium perfringens type C Isolates from Switzerland. In preparation, Dissertation, Vetsuisse Fakultät, Universität Bern

Project *1.12.05*

Project duration *01.02.2012 – 30.09.2013*