Background:

*Clostridium difficile* is the leading pathogen of hospital-acquired diarrhoea in human. The occurrence and clinical importance was also recognized in several animal species. We aimed to determine the occurrence of *C. difficile* in pigs in the Czech Republic with detail characterisation of isolates.

Material/methods:

A total of 198 pig diarrhoeal stool samples (49 swine and 149 piglets) from 23 farms distributed across the Czech Republic were cultured anaerobically on *C. difficile* selective media (Oxoid). *C. difficile* isolates were further investigated by capillary electrophoresis (CE) ribotyping, *tcdC* and *gyrA* gene fragment sequencing, multi-locus variable tandem-repeats analysis (MLVA) of seven loci (A6Cd, B7Cd, C6Cd, E7Cd, F3Cd, G8Cd, H9Cd) and for the presence of toxin genes (*tcdA*, *tcdB*, *cdtA*, *cdtB*). Antimicrobial susceptibility to metronidazole, vancomycin, tetracycline and moxifloxacin was determined by E- test strips (Liofilchem) on Wilkins Chalgren agar.

Results:

A total of 198 pig diarrhoeal stool samples from 23 farms were investigated and 58 samples (56 piglets, 2 swine) from 12 farms were *C. difficile* positive. A total of 58 isolates were further characterised. CE-ribotyping revealed nine different profiles (011 n=5, 014 n=1, 033 n=10, 078 n=24, 078-variant n=5, 126 n=1, 150 n=7, 413 n=4, and new n=1). Isolates of ribotypes (RTs) 033, 078, 078-variant and 126 were positive for the presence of *cdtA* and *cdtB* (binary toxin) and harboured 39 bp deletion with substitution C184T in the *tcdC* gene. MLVA of selected isolates (n=30) revealed clonal
relatedness between isolates belonging to the RT078 (4 isolates from 2 litters and 7 isolates from 6 litters), RT078-variant (2 isolates, 1 litter), RT150 (3 isolates, 2 litters), RT049 (3 isolates, 1 litter), Figure 1. Each clonal complex corresponded to the different farm. All isolates were susceptible to metronidazole, vancomycin and tetracycline. Eleven isolates (RTs 150 n=7, 078-variant n=5) were moxifloxacin resistant (MIC≥32mg/L) and carried Thr82Ile in the GyrA.

Conclusions:

The high occurrence of *C. difficile* and different distribution of PCR ribotypes with the predominance of RT078 were observed in Czech piglets. Moreover, the clonal spread of moxifloxacin resistant *C. difficile* strains were found in two farms. The investigation of genetic relatedness between pig isolates from this study and Czech human *C. difficile* isolates from the Czech national collection is needed to determine the role of carriage of *C. difficile* in piglets and *C. difficile* infection in humans in the Czech Republic.

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