DYNAMIC, WHOLEGENOME SEQUENCING CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE PROFILES OF CLOSTRIDIOIDES DIFFICILE IN A PIG FARM FROM FARROWING TO WEANING

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Introduction

Clostridioides difficile is a zoonotic pathogen that has changed its epidemiology in the recent years (1).
Despite being associated with neonatal diarrhea in pigs and high antibiotic resistance (2,3), its transmission throughout time is still not well defined.

Objectives

This study describes the prevalence of *C. difficile* in a pig farm over the lactation period and the genetic and antimicrobial resistance characterization of the strains isolated.

Materials and methods

Sampling:

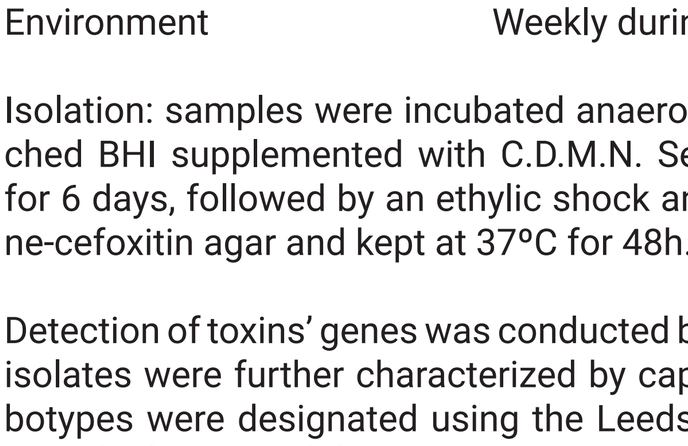
Digestive swabs from:	Temporal distribution:
8 farrowed sows	Before farrowing
5 piglets from each litter	Within 24-48h after parturition
Environment	Weekly during the following 3 weeks

Isolation: samples were incubated anaerobically at 37 ºC in pre-enriched BHI supplemented with C.D.M.N. Selective supplement (Oxoid) for 6 days, followed by an ethylic shock and then plated on cycloserine-cefoxitin agar and kept at 37ºC for 48h.

Detection of toxins' genes was conducted by a multiplex PCR. *C. difficile* isolates were further characterized by capillary ribotyping and PCR-ribotypes were designated using the Leeds-Leiden database. The antimicrobial susceptibility testing was carried out against seven antimicrobials by the Etest method.

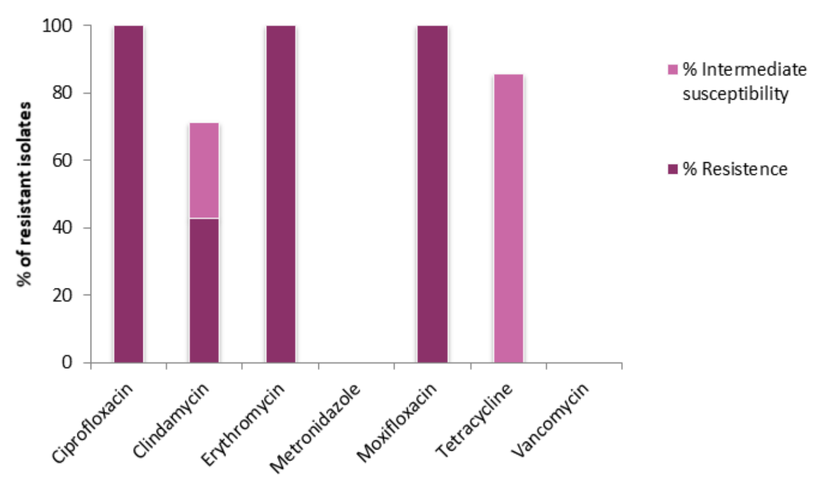
WGS was performed to assess the genetic relatedness. DNA was extracted with a commercial kit and analysed on an Illumina MiSeq sequencer and MinION. Genome sequences obtained from Illumina and MinION sequencing were analysed using the public Galaxy server at Wageningen University and Research centre (WUR) in The Netherlands (http://galaxy.wur.nl/)

Results



*Data from 2nd week of March may be altered due to Covid-19 lockdown measures during isolation.

The overall prevalence was 34.4% (95%; 29.4-39.4). The prevalence was high at farrowing, increased in the first week and decreased in the consecutive weeks. *C.difficile* was not isolated from sows during pregnacy, but it was detected 24-48h after farrowing in all of them.

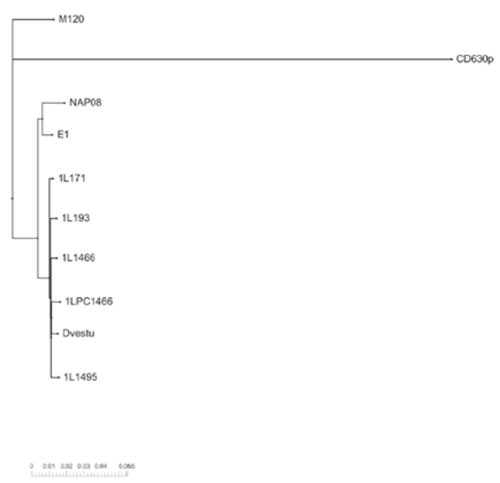


Prevalence of antimicrobial resistance in *C. difficile* isolates. No differences in antimicrobial resistance profiles were observed, regardless of the use of antimicrobial treatment.

Piglets	Diarrhoeic	Non-diarrhoeic	
C. diff postive	9	107	116
C. diff negative	28	202	230
	37	309	346

The proportion of piglets with diarrhea and *C. difficile* was similar to those infected but without symptoms (p=0.21).

Phylogenetic relationship between the whole genomes from the 6 C. difficile isolates analysed by WGS in this study and the genomes of *C. difficile* M120 (RT078), NAP08 (RT078), E1 (RT126) and CD630p (RT012) available on GenBank. The WholeGenome Sequencing (*WGS*) analysis showed clustering of isolates regardless the sample's origin.



Conclusions

1. *C. difficile* spreads rapidly within a pig farm and piglets (diarrheic or not) seem to play a significant role in its dissemination.
2. Farrowing seems to be a risk factor because pregnant sows with no *C.difficile* isolation became infected. Pregnancy-related immune suppression and environmental pressure of *C. difficile* may explain these findings.
3. The multiple antibiotic resistances among the isolates highlight the need to establish specific control strategies to prevent, from a One-Health approach, spreading of *C. difficile*.

References

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