MOLECULAR CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* ISOLATED FROM PIGS AND HUMANS IN NORTH-EASTERN ITALY

I. Drigo[1*], F. Agnoletti[1], C. Bacchin[1], E. Tonon[1], B. Marcon[1], P. Spigaglia[2], F. Barbanti[2], T. Ferro[1], M. Bonci[1], L. Bano[1]

[1] Istituto Zooprofilattico Sperimentale delle Venezie , Treviso, Italy
[2] Istituto Superiore di Sanità, Roma, Italy

* Corresponding author: idrigo@izsvenezie.it

OBJECTIVES. This study aimed to investigate the spread of *Clostridium difficile* (CD) among pigs in Italy and perform molecular characterization of CD of swine and human origin isolated in the same geographical area (North-East Italy) in order to investigate the circulation of genetically correlated strains

METHODS

Samples. 366 swine feces collected in 70 different piggeries and 119 CD positive stool samples collected in three hospitals were analyzed for the presence of CD.

Microbiological analysis. Each sample was pre-enriched on Taurocholate Cefoxitin Cycloserine Fructose (TCCF) Broth (1) and isolation was performed both in Columbia Agar Base added with aesculin and horse blood red cells (2) and TCCF Agar (3). Identification was based on morphological criteria and confirmed by MALDI-TOF (Bruker Daltonics) mass spectrometry.

Biomolecular characterization. Strains were characterized by *tcdA*, *tcdB* (4) and binary toxin genes detection (5) and by PCR-ribotyping (RT, 6) and toxinotyping (TT, 7). *TcdC* gene deletions (8) and antimicrobial resistance genes *tetM*, *tetW* (9), *erm B* (10) and AA substitution Thr82→Ile (*gyrA*), Asp426→Asn Val and Asp426→Val (*gyrB*) (11) were also investigated.

RESULTS

- 81/366 (22.1%)swine samples positive for CD
- highest prevalence in suckling piglets (fig. 1)
- 100 % of strains are toxigenic, the 42.5% of these carries binary toxin coding genes.
- 10 different RTs were identified, among these RT-150 TT 0, RT-078 TT V, RT-014/020 TT 0, RT-127 TT VI were the most frequently isolated (fig. 2).
- *ermB* and *tetM* genes were detected in 5.5% and 41.1% of the strains, no one resulted positive for *tetW.*
- 23.3% of the strains carried the AA substitution Thr82→Ile

- 109 strains
- 1.8% resulted non toxigenic
- binary toxin genes were detected in 8.4% of toxigenic strains
- 15 different ribotypes have been identified, among these, RT-018 TT 0, RT-014/020 TT0, RT-001 TT 0 and RT-126 TT V were the most prevalent (fig. 3)
- *ermB* and *tetM* genes were detected in 11.9% and 8.5% of the isolates, no one resulted positive for *tetW*
- 73.4% carried the AA substitution Thr82→Ile in *GyrA* and only the 0.9% the Asp426→Asn in *GyrB*

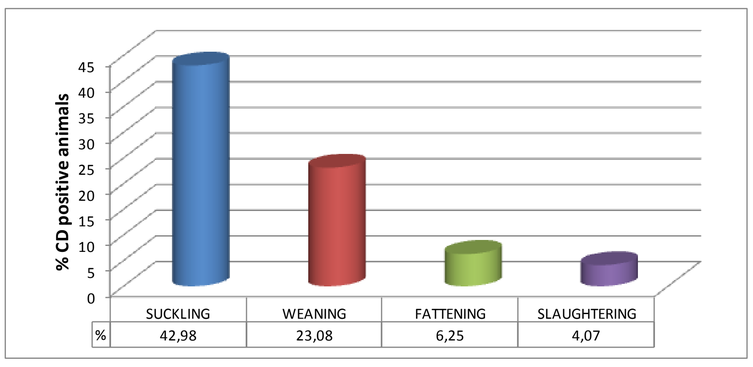

Figure 1. Percentage of swine positive for CD divided in 4 age groups.

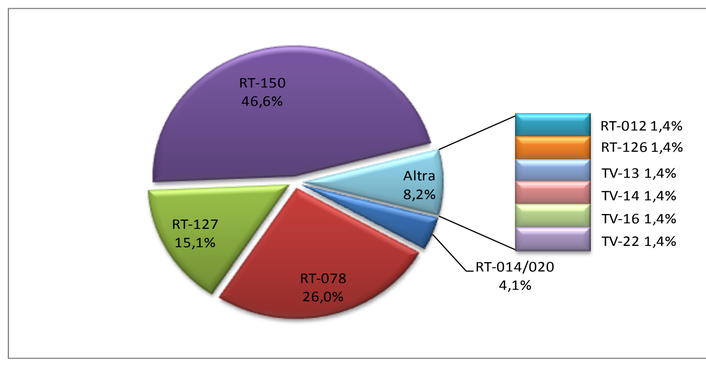

Figure 2. PCR ribotypes isolated in swine

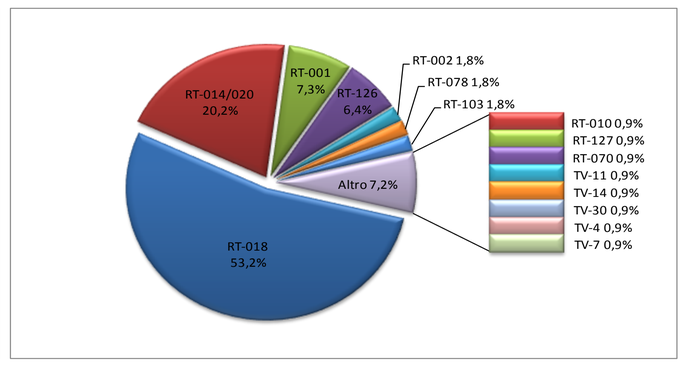

Figure 3. PCR ribotypes isolated in humans

CONCLUSIONS

- CD is widespread in Italian piggeries and, as previously reported in other studies the percentage of positivity decreases in a age-depending manner.
- RT-078 TT V (12/70 piggeries) and RT-150 TT O (9/70 piggeries) are the most prevalent RTs in Italian pigs
- Only one RT has been isolated in each farm indicating a clonal spread of the strain among each single productive unit
- *TetM* gene is widespread in swine strains and most of positive strains belong to RT-078
- 5/10 CD RTs (014/020, 126, 078, 127 and TV14) isolated from swine have been also isolated from humans
- RT-018 TT 0 is confirmed as the most prevalent CD type in human in Italy but it was not detected in pigs
- A high percentage of human isolates shows the AA substitution Thr82→Ile and most of these strains belong to RT-018

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