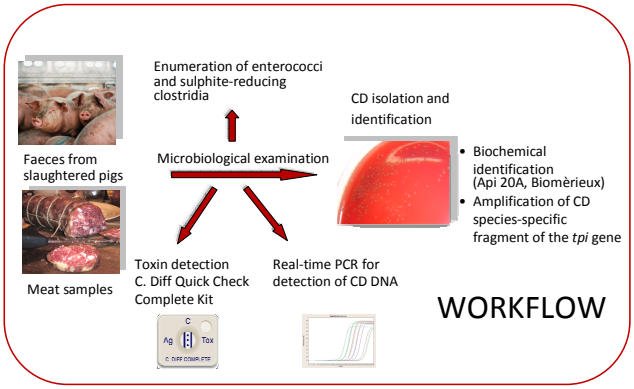
Clostridium difficile in locally produced salami in North-Eastern Italy

Drigo I., Tonon E., Bacchin C., Bano L., Bonci M., Cereser A., Pezzuto A., Favretti M., Furlan F., Agnoletti F.

Istituto Zooprofilattico Sperimentale delle Venezie, Treviso, Italy

BACKGROUND

Community-associated *Clostridium difficile* infection (CA-CDI) has emerged as a topic of interest since, recently, *C. difficile* infections (CDI) have been increasingly reported worldwide in populations previously though to be at low risk, i.e. persons not exposed to health care settings or without history of antibiotic treatments, young people and pregnant women (1-3). *C. difficile* (CD) is widely distributed in the environment and recovered from a broad range of animals included food animals (4). Recently, it was demonstrated that its spores can also be detected in meat products. The characterization of CD strains isolated from retail meat showed that they belonged to PCR ribotyope 027 toxinotype III and 078 toxinotype V which are two of the most important type strains involved in human infections (5-9). Among these ribotypes the latter is of particular concern, given that it is one of the most frequently isolated strains in CA-CDI worldwide and that it has also been extensively isolated in food animals both in USA and in Europe (10). These findings raised the hypothesis that meat products could be a source of CD for human infection. A recent cross-sectional study conducted in Italy in 2009 on samples of meat purchased from 150 retail outlets demonstrated that ground beef and pork sold at retail in North-Eastern Italy do not represent a risk factor for human CDI (11).
This study aimed to evaluate the presence of CD spores in locally produced pork sausages and salami in Treviso and Venice provinces.



MATERIAL AND METHODS

Samples

In total 416 meat samples were analyzed: 152 fresh spiced minced pork, 81 fermented salami after a mean seasoning of 20 days and 183 salami after mean seasoning of 60 days. The presence of CD was evaluated also in faecal samples obtained from the slaughtered pigs.

Microbiological analysis

Each sample was added to CD selective medium Taurocholate Cefoxitine Cycloserine Fructose Broth (ratio 1 to 9) (Oxoid) and incubated at 37 °C for ten days in anaerobic conditions (90 % N_2, 5% CO_2 and 5% H_2). After incubation the broth cultures were homogenized and alcohol shock was performed at room temperature by mixing 2 mL of the homogenized broth with 96% ethanol (1:1 v/v) for 1 hour. The samples were subsequently centrifuged at 3800 x g for 10 min and the sediment was streaked onto Columbia Blood Agar Base (Oxoid) supplemented with aesculin (0.1% v/v) and horse red blood cells (5% v/v) and incubated for 48-72 hours in anaerobic chamber. A positive control consisting of meat experimentally infected with CD spores was carried out simultaneously for each batch of samples.

Identification of presumptive CD colonies.

The suspected colonies (brown-grey, non swarming, non haemolytic, rough and with typical horse manure-like smell) were subcultured and identification was carried out both by biochemical commercial kits (Api 20A, Biomèrieux) and by PCR amplification of CD species-specific fragment of the *tpi* gene (12).

Further samples analysis

To confirm the microbiological evaluation, the presence of CD and its A and B toxins in the broth culture supernatants was evaluated by C. DIFF QUIK CHECK COMPLETE KIT (Techlab). The presence of CD DNA was also assessed by real-time PCR targeting on specific fragment of CD 16S rDNA (13).
Enumeration of enterococci and sulphite-reducing Clostridia (ISO7937:2004) was also performed to the purpose of estimating the correlation between CD positivity and faecal contamination during meat processing.

RESULTS

- None of the 416 analyzed meat products were positive for CD by microbiological and PCR analysis and the detection of toxin A and B in the supernatant cultures gave negative results as well.
- Clostridial spores and enterococci counts differed greatly in magnitude between samples.
- Five over 123 faecal samples collected from slaughtered animals resulted positive for toxigenic CD.

CONCLUSION

The low percentage of faeces positive for CD (4%) obtained from slaughtered swine is consistent with the low prevalence of this microorganism in grower-finisher pigs reported in previously published studies. The low carriage rate within finishing pigs and proper meat handling procedures seem to make pork products at low risk for CD transmission into the food chain.

References

1. Abrahamian FM *et al*. Update on emerging infections from the Centers for Disease Control and Prevention. severe *Clostridium difficile*-associated disease in populations previously at low risk: four states, 2005. Ann Emerg Med. 2006;48(1):55-9.
2. Wilcox M *et al*. A case-control study of community-associated *Clostridium difficile* infection. J Antimicrob Chemother. 2008; 62:388-96.
3. Rupnik M *et al*. *Clostridium difficile* infection: New developments in epidemiology and pathogenesis. Nat Rev Microbiol. 2009;7(7): 526-36.
4. Indra A *et al*. *Clostridium difficile*: A new zoonotic agent? Wien Klin Wochenschr. 2009;121(3-4): 91-5.
5. Rodriguez-Palacios A *et al*. Possible seasonality of *Clostridium difficile* in retail meat, Canada. Emerg Infect Dis. 2009;15(5): 802-5.
6. Rodriguez-Palacios A *et al*. *Clostridium difficile* in retail ground meat, Canada. Emerg Infect Dis. 2007;13(3): 485-7.
7. Songer JG *et al*. *Clostridium difficile* in retail meat products, USA, 2007. Emerg Infect Dis. 2009;15(5): 819-21.
8. Von Abercron SM *et al*. Low occurrence of *Clostridium difficile* in retail ground meat in Sweden. J Food Prot. 2009;72(8): 1732-4.
9. Weese JS *et al*. Detection and enumeration of *Clostridium difficile* spores in retail beef and pork. Appl Environ Microbiol. 2009;75(15): 5009-11.
10. Dawson LF *et al*. *Clostridium difficile*: a continually evolving and problematic pathogen. Infect Genet Evol. 2009;9(6): 1410-7.
11. Drigo I *et al*. Prevalence of *Clostridium difficile* in retail meat products in North-Eastern Italy. 20th ECCMID. Clin. Microbiol. Infect. 2009; 16 (2): S38.
12 Lemee, L. *et al*. Multiplex PCR targeting *tpi* (triose phosphate isomerase), *tcdA* (toxin A), and *tcdB* (toxin B) genes for toxigenic culture of *Clostridium difficile*. J. Clin. Microbiol. 2009; 42: 5710-5714.
13. Penders, J. *et al*. Quantification of *Bifidobacterium spp.*, *Escherichia coli* and *Clostridium difficile* in faecal samples of breast-fed and formula-fed infants by real-time PCR. FEMS Microbiol. Lett. 2005; 243: 141-147.