CLOSTRIDIUM DIFFICILE IN PIGS AND CATTLE AT SLAUGHTERHOUSE, CARCASS CONTAMINATION AND PREVALENCE IN RETAIL MEAT IN BELGIUM

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**Clostridium difficile** associated disease

- Gram-positive anaerobic spore-forming bacterium recognized as the major cause of hospital-acquired diarrhea and colitis associated with antibiotic therapy\(^1\)

- Toxin A and B are major virulence factors\(^2\)

- Exposure to antibiotics: clindamycin, cephalosporins, fluoroquinolones\(^3\)

- Emergence of a hyper-virulent strain PCR-ribotype 027 associated with increased morbidity and mortality\(^2\)

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\(^2\)Warny et al., 2005. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. The Lancet, 366, 1079-84.

\(^3\)Pepin et al., 2005. Emergence of Fluoroquinolones as the Predominant Risk Factor for *Clostridium difficile*–Associated Diarrhea: A Cohort Study during an Epidemic in Quebec. Clin Infec Dis., 41,1254? 60.
Epidemiology of *Clostridium difficile*

1Barbut et al., 2009. Prospective study of *Clostridium difficile* infections in Europe with phenotypic and genotypic characterisation of the isolates. Clin Microbiol Infect, 13, 1048-57.

1Delmée, M., 2012. Epidemiology of *Clostridium difficile* in Belgium. NRC *Clostridium difficile*-Yersinia.
Emergence of community-acquired 
*Clostridium difficile* infection

- Emerging data on the occurrence of *C. difficile* infection in non-hospitalized patients
- Absence of traditional risk factors
- Less severe diarrhea (mild/moderate) and protracted
- Successful treatment with metronidazole

**Clostridium difficile in animals and food**

- In animals, *C. difficile* appears to be an important cause of enteric disease.

- Asymptomatic carriage of *C. difficile* in animals has been also described.

- *C. difficile* has been recently isolate from a variety of meat products.

- *C. difficile* meat isolates are correlated with the types implicated in human disease.
Hypothesis about a potential risk of foodborne infections linked to *Clostridium difficile*
Objectives

• Determine the presence of *C. difficile* in young animals on farms

• Determine the presence of *C. difficile* in intestinal contents and on carcasses in full-grown animals at the slaughterhouse

• Evaluate the presence of *C. difficile* in retail meat sold in market places in Belgium

• Characterize the isolates by PCR-ribotype, presence of toxin genes and toxigenic activity in order to compare the strains with the main PCR-ribotypes found in humans in Belgium
Study design

Farm animals

From January to July 2011

- Piglets faecal samples
  - 23 new-born pigs (still suckling <15 days old)
  - 3 different breeding farms
  - Piglets without diarrhea

- Calves faecal samples
  - 18 non-diarrhoeic calves (<3 months of age)
  - 5 different local farms
  - Clinically healthy calves
Study design

Slaughter animals

*From January to July 2011*
(9 different visits to a local slaughterhouse)

- **Pigs intestinal samples**
  - 194 samples from pigs
- **Cattle intestinal samples**
  - 202 samples from cattle
Study design

Intestinal contents and carcass samples

From September to December 2011

9 different visits to a local slaughterhouse

- **Pigs**
  - Intestinal samples (n= 100)
  - Carcasses from pigs (n=100)
  - Intestinal contents and carcass swabs were taken from different animals

- **Cattle**
  - Intestinal samples (n= 101)
  - Carcasses from pigs (n=101)
  - 80.1% carcass and intestinal samples were taken from the same animal

Belgian Royal Decree of 20 August 2002
Study design

Meat samples

*From January to June 2012*

- **Beef samples (n=133) and pork samples (n=107)**
  - 21 different retailers were visited
  - 5-18 samples from pork and beef were collected weekly (one beef and pork sample by establishment)
  - Each establishment was visited during at least three different weeks

Pure pork, pure beef, pure pork or pure beef burgers and sausages were purchased
Methodology

- **Direct and enrichment culture**
  Home-made cycloserine cefoxitin fructose taurocholate
  (Delmée et al., 1987. Epidemiology and prevention of *Clostridium difficile* in a leukaemia unit. E J Clin Microbiol, 6, 623-27)

- **C. difficile** latex agglutination rapid test Kit DR 1107A Oxoid
- Detection of a species-specific internal fragment of *tpi*, detection of genes for toxin B, toxin A and binary toxin (*cdtA*) by PCR et Genotype Cdiff test system
  (Lémée et al., 2004. Multiplex PCR targeting *tpi* (triose phosphate isomerase), *tcdA* (toxin A), and *tcdB* (toxin B) genes for toxigenic culture of *Clostridium difficile*. J Clin Microbiol, 42, 5710-14)
  (Antikainen et al., 2009. Detection of virulence genes of *Clostridium difficile* by multiplex PCR. Acta Phat, Microbiol Inmuno Scand, 117, 607-13)

- **Cytotoxicity assay using confluent monolayer MRC-5 cells**
  Cytotoxic activity was confirmed using a specific *C. difficile* antitoxin kit (T500, TechLab, Virginia, USA)
  (Rodriguez et al., 2012. *Clostridium difficile* in young farm animals and slaughter animals in Belgium. Anaerobe, 18, 621-625)

- **PCR-ribotyping**

- **Multilocus Sequencing typing**
Results: Prevalence of *C. difficile* in farm and slaughter animals

*C. difficile* recovery from faecal samples:

**Farms**

- 4/18 samples from calves (22.2%)
- 18/23 samples from piglets (78.3%)

**Slaughterhouse**

- 4/202 samples from slaughter cattle (6.9%)
- 0/194 samples from slaughter pigs (0%)

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**Pathogenesis and toxins**

*Clostridium difficile* in young farm animals and slaughter animals in Belgium

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Results: Prevalence of *C. difficile* in slaughter animals and carcass contamination

*C. difficile* recovery:

### Intestinal contents
- 10/101 samples from cattle (9.9%)
- 1/100 samples from pigs (1%)

### Carcass samples
- 8/101 samples from cattle (7.9%)
- 7/100 samples from pigs (7%)
Results: Prevalence of *C. difficile* in retail meat in Belgium

**Beef samples**

3/133 samples (2.3%)

**Pork samples**

5/107 samples (4.7%)

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Isolate number</th>
<th>PCR-ribotype</th>
<th>Toxin activity</th>
<th>Detection of toxin genes by PCR</th>
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<tbody>
<tr>
<td></td>
<td>number</td>
<td></td>
<td></td>
<td>tcdA</td>
</tr>
<tr>
<td>Retail Beef</td>
<td>Organic beef burger</td>
<td>2404</td>
<td>078</td>
<td>+</td>
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<tr>
<td>Beef burger</td>
<td>2001</td>
<td>014</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ground beef</td>
<td>3030</td>
<td>014</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Retail Pork</td>
<td>Organic Chipolata</td>
<td>2405</td>
<td>078</td>
<td>+</td>
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<tr>
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<td>+</td>
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<tr>
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<td>014</td>
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<td>UCL378</td>
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</tr>
</tbody>
</table>

1. Presence of deletions in the regulator gene tcdC
2. Presence of mutation in the gyrA gene associated with moxifloxacin resistance
Discussion: *C. difficile* in farm and slaughter animals

**Farm**

- **Prevalence 78.3%**
  - Main PCR-ribotypes: 078/002

- **Prevalence 22.2%**
  - Main PCR-ribotypes: 078/UCL46

- **Prevalence 0-1%**
  - Main PCR-ribotypes: 078/UCL46

- **Prevalence 6.9-9.9%**
  - Main PCR-ribotypes: 078/ Great variety of types (UCL5, 014, 002)

**Slaughterhouse**

Similar prevalences and types previously reported in other countries as Canada, The Netherlands, Slovenia or Spain


Avbersek et al., 2009. Diversity of *Clostridium difficile* and other animals in Slovenia. Anaerobe, 15, 252-5.

Discussion: *C. difficile* on pigs and cattle carcasses

Prevalence 7%
Main PCR-ribotypes 014/081/UCL36

Prevalence 7.9%
Great variety of types (UCL5a/UCL16u)

Similar studies describing *C. difficile* on pig and cattle carcasses at the slaughterhouse in North America and Canada

First isolation in Europe

Susick et al., 2012. Longitudinal study comparing the dynamics of *Clostridium difficile* in conventional and antimicrobial free pigs at farm and slaughter. Vet Microbiol 25, 172-78.

Rodriguez-Palacios et al., 2011. Transient faecal shedding and limited animal-to-animal transmission of *Clostridium difficile* by naturally infected finishing feedlot cattle. Appl Envir Microbiol 77, 3391-97.


Discussion: *C. difficile* in retail meat

**Prevalence 2.3%**
Main PCR-ribotypes 078/014

**Prevalence 4.7%**
Main PCR-ribotypes 078/014/UCL57

**Prevalence of *C. difficile* previously reported in meat**
- **America**: 1.8 - 20% of positives
- **Europe**: 3% of positives
- Main PCR-ribotypes in America 078 and 027

First isolation of PCR-Ribotypes 078 and 014 in retail meats in Europe

Discussion: Ribotypes distribution in Belgian hospitals

- In 2011 in Belgium, the most prevalent PCR ribotypes in hospitals were:
  - 014***, 002*, 027, 078***, 020, UCL46*, UCL161*, UCL26, 001, 023*, UCL23f, 012, UCL16b, 015*, UCL5a**, UCL20a*, and UCL49 sorted by decreasing values in number of isolates.

Intestinal contents  Carcasses  Meat

- Overlap of PCR-ribotypes isolated from meat and human samples (MLST)

\(^{1}\)Delmée, M., 2012. Epidemiology of *Clostridium difficile* in Belgium. NRC *Clostridium difficile*-Yersinia.
In Progress

• Nursing Home Study

OBJECTIVE:

• To evaluate and follow the prevalence of *C. difficile* in a Belgian nursing home.

METHODOLOGY:

• During a 4-month period, stool samples from a group of 23 elderly care home residents were collected weekly.

• A *C. difficile* microbiological detection scheme was performed along with an overall microbial biodiversity study of the faeces content by Targeted Metagenomic analysis.
Conclusions

• This study further documented that animals are carriers of *C. difficile* at slaughter, and carcass contamination occurs inside the slaughterhouse

• Toxigenic *C. difficile* is present in the slaughterhouse and in retail meat in Belgium

• Carcasses were contaminated with a variety of PCR-ribotypes suggesting a slaughterhouse environmental contamination and/or animal reservoir

• In meat samples, the PCR-ribotypes 014 and 078 were the most frequently identified. These ribotypes were also isolated from intestinal and carcass samples

• The results obtained prove that toxigenic *C. difficile* is present in ground meat in retail outlets in Belgium. However, the clinical relevance of ingesting spores of *C. difficile* with food needs further investigation
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