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

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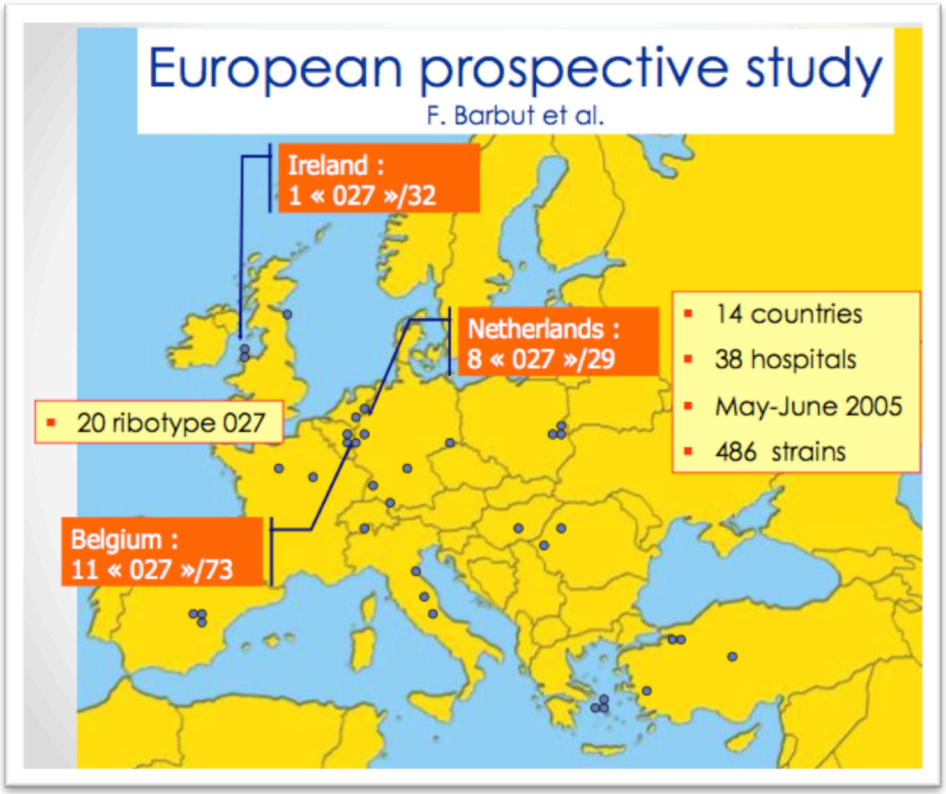
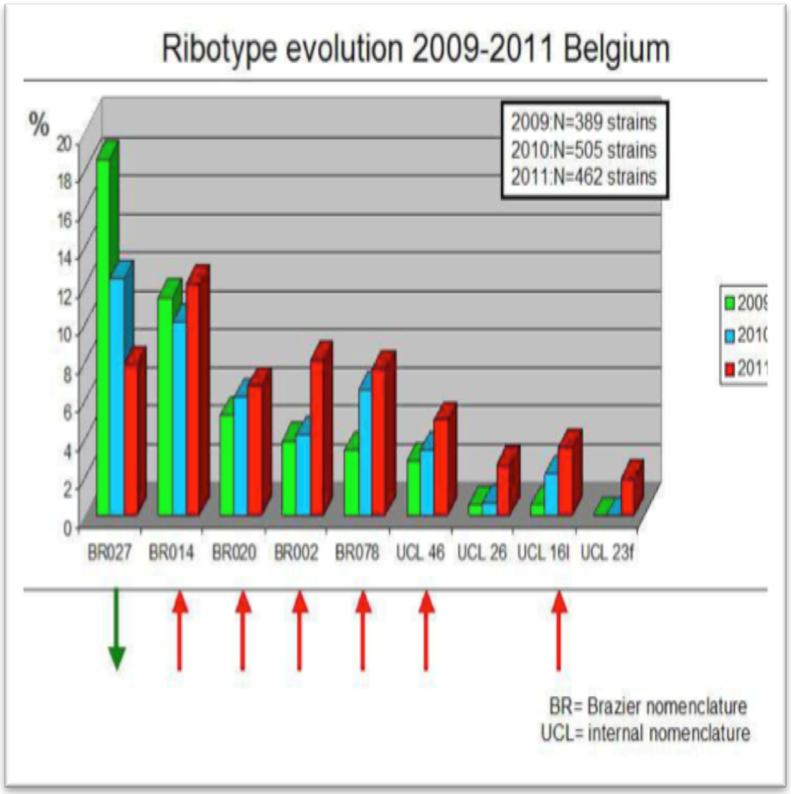
Clostridium difficile associated disease

- 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]

[1]Rupnik et al., 2009. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol., 7, 526-36
[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*





[1]Barbut 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.

[1]Delmée, 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

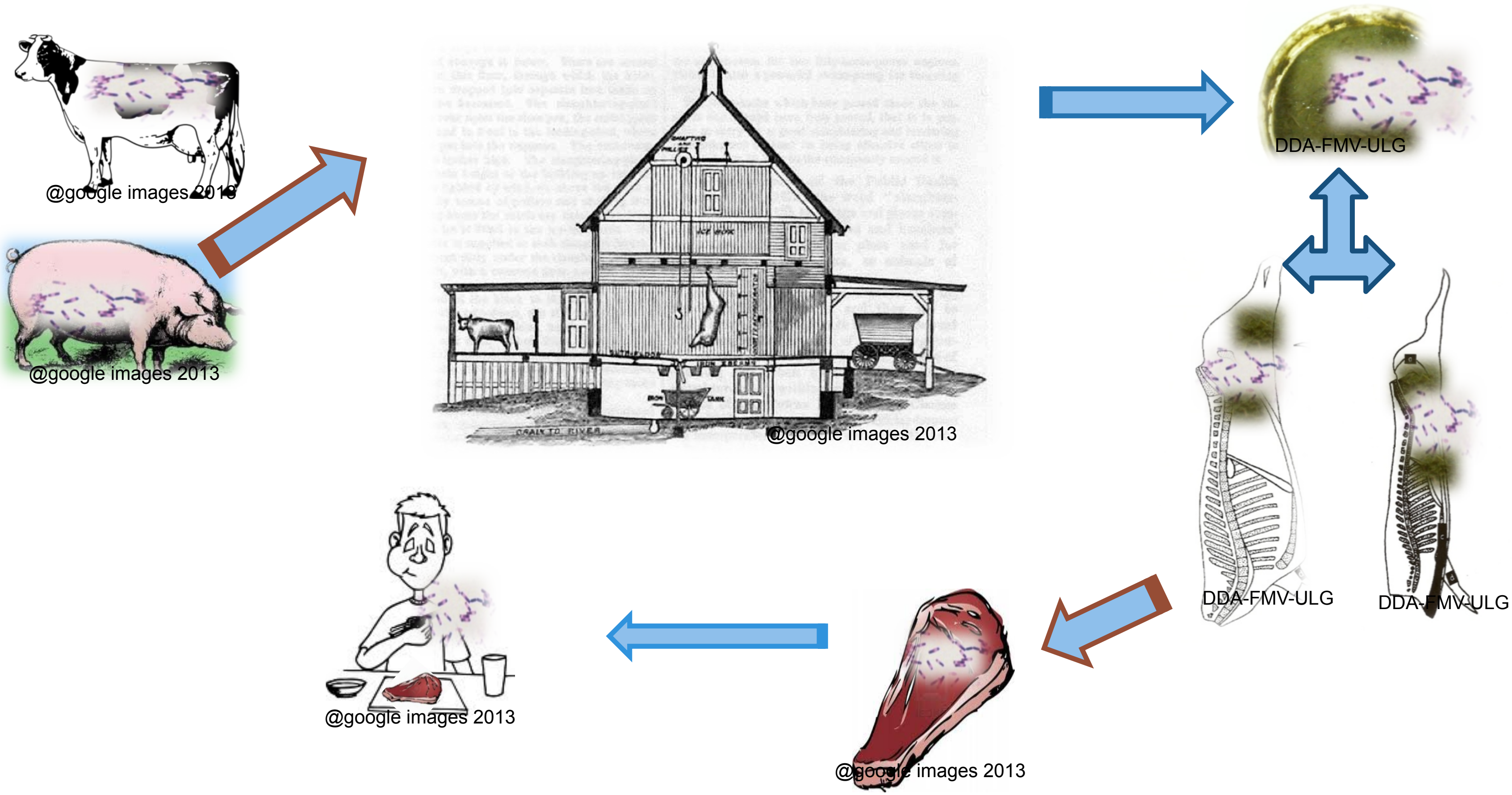
- Less severe diarrhea (mild/moderate) and protracted

- Successful treatment with metronidazole

Riley et al., 1995. Community acquired *Clostridium difficile* associated diarrhea. Clin Infect Dis., 20, 263-65.

***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

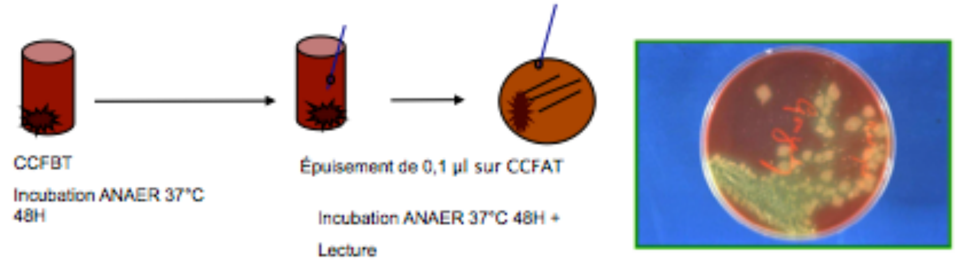
- **Beef samples (n=133) and pork samples (n=107)**
  - 21 different retailers were visited
  - 5-18 samples from pork and beef were collected weekly (one beef and pork sample by establishment)
  - Each establishment was visited during at least three different weeks Pure pork, pure beef, pure pork or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

  (Lemée 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**

  (Bidet et al.,1999. Development of a new PCR-ribotyping method based on ribosomal RNA gene sequencing. FEMS Microbiol Letters, 175, 261-66)

- **Multilocus Sequencing typing**

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

PCR-ribotypes and toxin gene profiles of *Clostridium difficile* isolated from food animals.

Animal group	PCR-ribotype	No. isolates	Cytotoxicity assay	Detection of toxin genes by PCR		
				tcdA	*tcdB*	*cdtA*
Piglets	078	12	+	−	+	+
	002	3	+	+	+	−
	172 UCL	1	+	−	+	+
	239 UCL	1	+	−	+	+
	9 UCL	1	−	−	−	−
Calves	078	3	+	−	+	+
	015	1	+	+	+	−
Cattle	002	1	+	+	+	−
	014	1	+	+	+	−
	081	1	+	+	+	−
	087	1	+	+	+	−
	118 UCL	1	+	+	+	−
	16l UCL	1	+	+	+	−
	16r UCL	1	+	+	+	−
	118a UCL	2	+	+	+	−
	20a UCL	1	+	+	+	−
	238 UCL	1	−	−	−	−
	103 UCL	1	−	−	−	−
	36 UCL	1	−	−	−	−
	273 UCL	1	−	−	−	−

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%)

PCR-ribotypes and toxin gene profiles of *Clostridium difficile* isolated from cattle and pigs intestinal contents, car

Animal group	Sample type	PCR-ribotype	No. isolates	Toxin activity	Detection of toxin genes by PCR		
					tcdA	*tcdB*	*cdtA*
Cattle	Intestinal contents	078	6	+	+	+	+
		UCL5a	1	+	+	+	+
		014	1	+	+	+	−
		UCL16L	1	+	+	+	−
		029	2	+	+	+	−
		UCL118	2	+	+	+	−
		UCL16R	1	+	+	+	−
		UCL254	2	+	+	+	−
		UCL270	1	−	−	−	−
		UCL273	1	−	−	−	−
		UCL103	2	−	−	−	−
	Carcass samples	023	2	+	+	+	+
		UCL5a	4	+	+	+	+
		UCL11	2	+	+	+	+
		015	2	+	+	+	−
		UCL16u	4	+	+	+	−
		UCL23d	1	+	+	+	−
Pork	Intestinal contents	078	1	+	+	+	+
		UCL46	1	+	+	+	−
	Carcass samples	014	7	+	+	+	−
		081	2	+	+	+	−
		UCL36	4	−	−	−	−

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Presence of *Clostridium difficile* in pigs and cattle intestinal contents and carcass contamination at the slaughterhouse in Belgium

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Results: Prevalence of *C. difficile* in retail meat in Belgium

Beef samples

3/133 samples (2.3%)

Pork samples

5/107 samples (4.7%)

PCR-ribotypes, toxin activity and gene profile of *Clostridium difficile* isolated from retail meat samples.

Sample type		Isolate number	PCR-ribotype	Toxin activity	Detection of toxin genes by PCR						
					tcdA	*tcdB*	*cdtA*	*tcdA*	*tcdB*	*cdtA*	*cdtB*
Retail Beef	Organic beef burger	2404	078	+	+	+	+	+	+	+	+
	Beef burger	2001	014	+	+	+	-	+	+	-	-
	Ground beef	3030	014	+	+	+	-	+	+	-	-
Retail Pork	Organic Chipolata	2405	078	+	+	+	+	+	+	+	+
	Ground pork	2012	014	+	+	+	-	+	+	-	-
	Ground pork	2403	014	+	+	+	-	+	+	-	-
	Pork Sausage	1003	UCL57	+	+	+	-	+	+	-	-
	Pork Sausage	1703	UCL378	-	-	-	-	-	-	-	-

[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

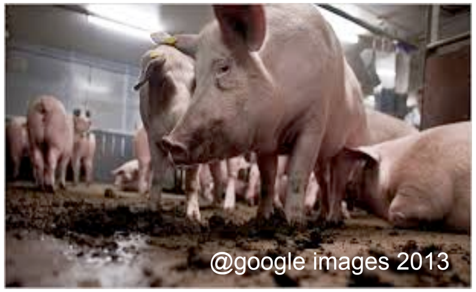
Farm

Prevalence 78.3%

Main PCR-ribotypes 078/002

Prevalence 22.2%

Main PCR-ribotypes 078/015



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

Costa et al., 2011. *Clostridium difficile* on a veal farm: prevalence, molecular characterization and tetracycline resistance. Vet Microbiol,152,379-84.
Keessen et al., 2011. The relation between farm specific factors and prevalence of *Clostridium difficile* in slaughter pigs. Vet MicrobiolL,154,130-4.
Hopman et al., 2011. Acquisition of *Clostridium difficile* by piglets. Vet Microbiol, 21,186-92.
Avbersek et al., 2009. Diversity of *Clostridium difficile* and other animals in Slovenia. Anaerobe, 15, 252-5.
Alvarez-Perez et al., 2009. Prevalence of *Clostridium difficile* in diarrhoeic and non-diarrhoeic piglets. Vet Microbiol, 137, 302-5.

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

Prevalence 7%

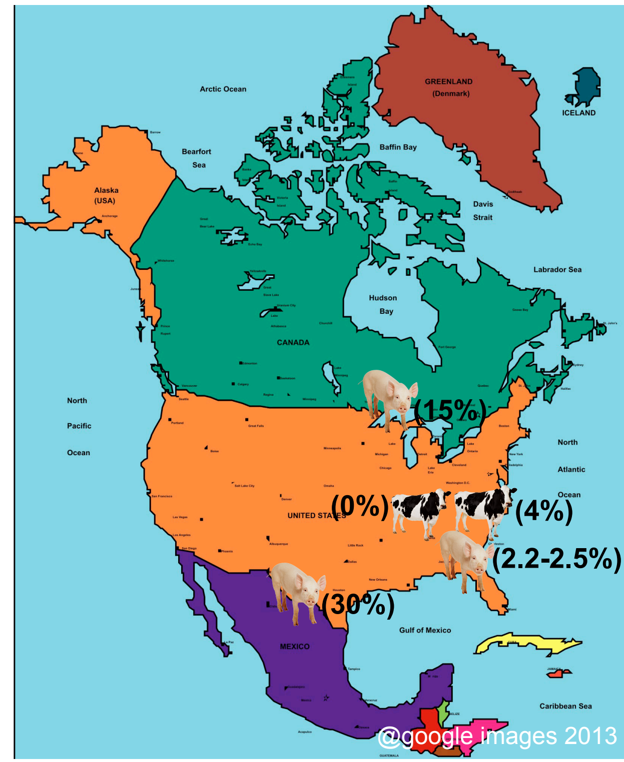
Main PCR-ribotypes 014/081/UCL36

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

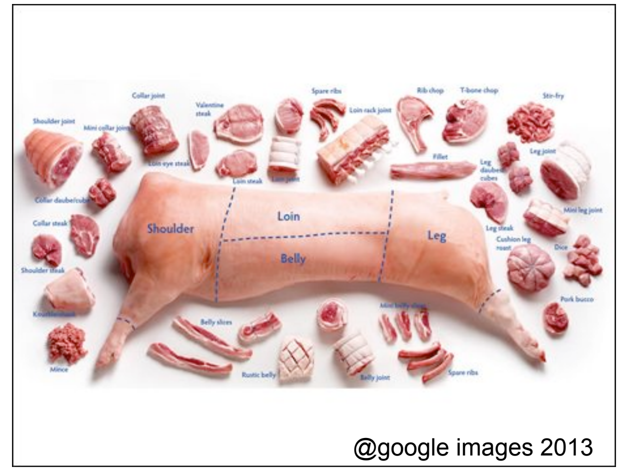


Susick et al., 2012. Longitudinal study comparing the dynamics of *Clostridium difficile* in conventional and antimicrobial free pigs at farm and slaughter. Vet Microbiol 25, 172-78.
Rodriguez-Palacios et al., 2011. Transient faecal shedding and limited animal-to-animal transmission of *Clostridium difficile* by naturally infected finishing feedlot cattle. Appl Envir Microbiol 77, 3391-97.
Harvey et al. , 2011. *Clostridium difficile* in retail meat and processing plants in Texas. Journal of Veterinary diagnostic investigation 23, 807-11.
Hawkin,et al.,, 2012. Carriage and dissemination of *Clostridium difficile* and methicillin resistant *Staphylococcus aureus* in pork processing. Food Control, 31,433-37.
Houser, et al., 2012. Prevalence of *Clostridium difficile* toxin genes in the faeces of veal calves and incidence of ground veal contamination. Foodborne Path dis., 9, 32-6.

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

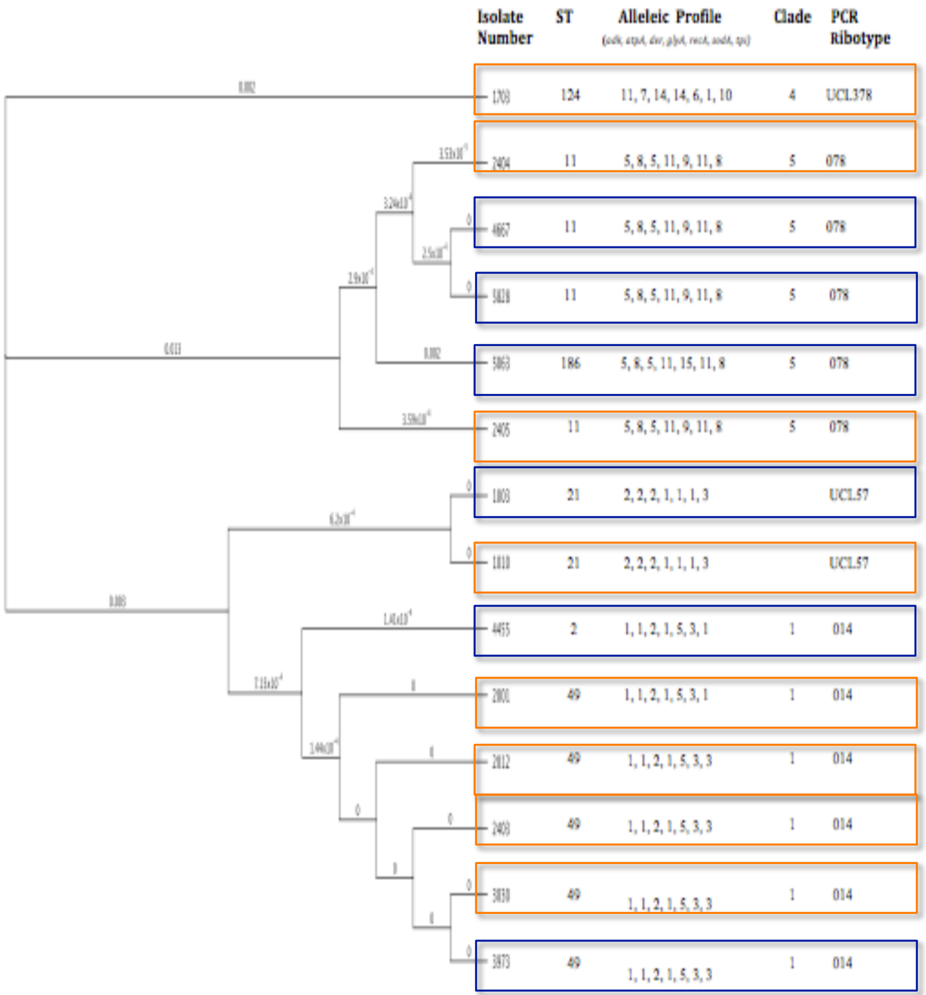
Hensgens et al., 2012. *Clostridium difficile* infection in the community: a zoonotic disease? Clin Microbiol Infec Dis 18, 635-45.

Discussion: Ribotypes distribution in Belgian hospitals

- In 2011 in Belgium, the most prevalent PCR ribotypes in hospitals were
  - **014*****, **002***, **027**, **078*****, **020**, **UCL46***, **UCL16I***, **UCL26**, **001**, **023***, **UCL23f**, **012**, **UCL16b**, **015***, **UCL5a****, **UCL20a***, and **UCL49** sorted by decreasing values in number of isolates.

Intestinal contents [purple] **Carcasses** [green] **Meat** [orange]

- 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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