Clostridium difficile: an emerging zoonotic pathogen

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Diagnostic et surveillance des maladies infectieuses

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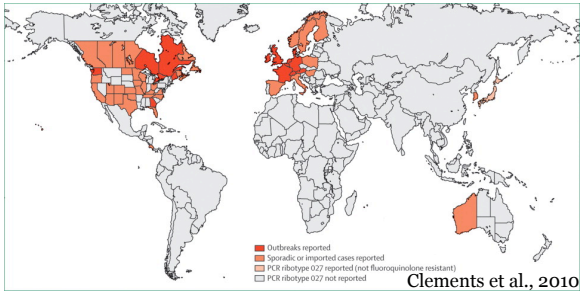
Centre culturel de Woluwé-St-Pierre

Background

- Since toxigenic *C. difficile* was recognized as the major cause of antibiotic-associated diarrhea and pseudomembranous colitis in 1978, many outbreaks have been documented

- In the last years, an enhanced virulence and increased antibiotic resistance of *C. difficile* strains (PCR-ribotype 078/NAP-1/B1) has been observed



Clements et al., 2010

- There are emerging data on the occurrence of *C. difficile* infection in the community: non-hospitalized and younger patients with absence of other traditional risk factors

Hypothesis about a potential risk of foodborne infections linked to *C. difficile*

https://www.health.harvard.edu

Background

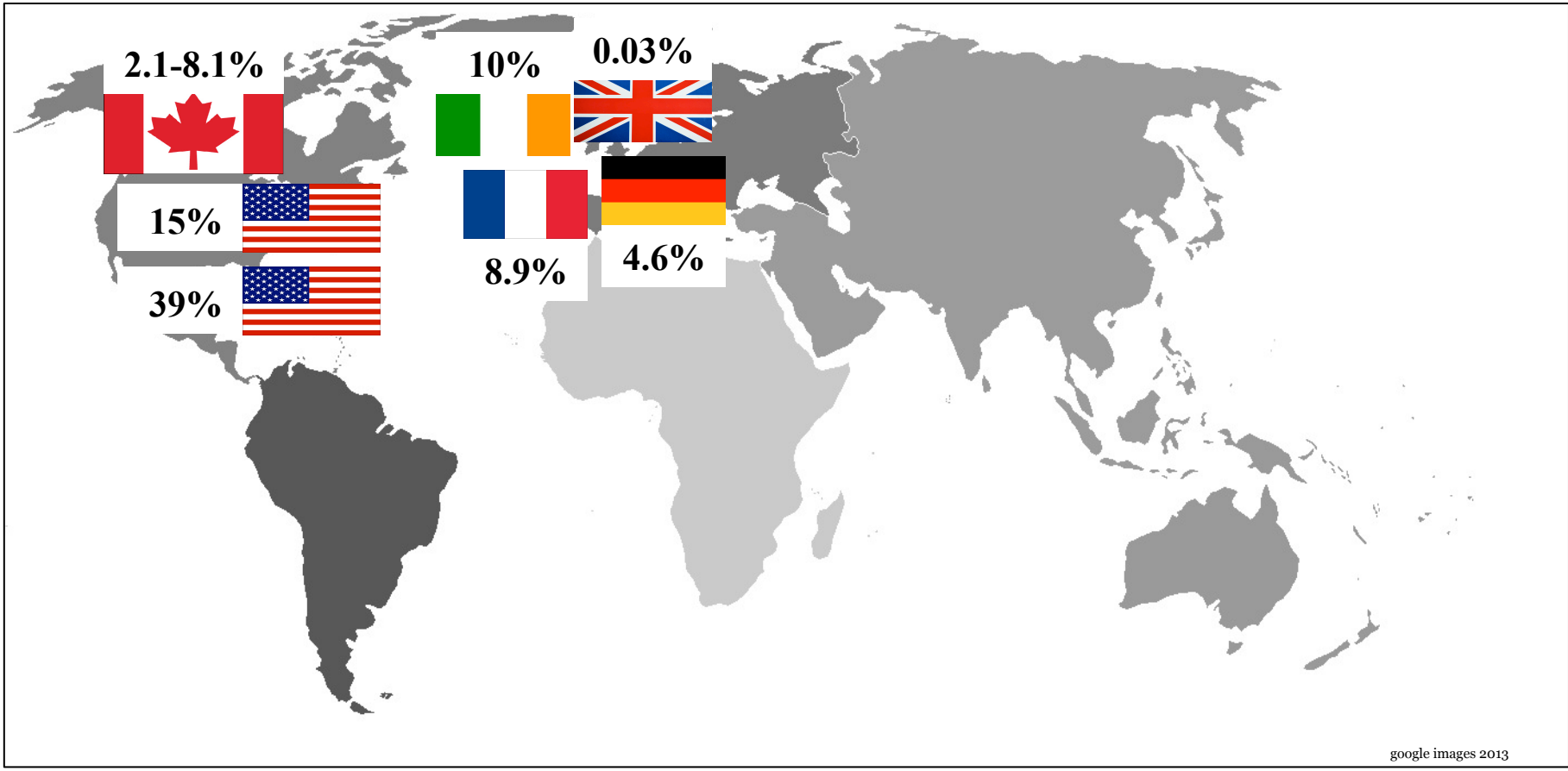
- Patients with serious illnesses and prolonged hospitalizations are at particular risk, as people above 65 years of age

- The increased risk of acquiring *C. difficile* in the elderly may be due to age-related changes in intestinal flora, immune senescence or the presence of underlying diseases

- There is not much data describing the prevalence and molecular epidemiology of *C. difficile* in nursing homes in absence of an epidemic situation

Arvand, M., et al., 2012. High prevalence of *Clostridium* difficile colonization among nursing home residents in Hesse, Germany. Plosone, 7, e30183.

Clostridium difficile Infection in Nursing homes



Arvand, M., et al., 2012. High prevalence of *Clostridium* difficile colonization among nursing home residents in Hesse, Germany. Plosone, 7, e30183.

Birgand, G., et al., 2010. Investigation of a large outbreak of *Clostridium diff*icile PCR-ribotypes 027 infections in northern France, 2006-2007 and associated clusters in 2008-2009. Eurosur, 24, 1-6.

Marwick, C.A., et al 2013. Community-associated *Clostridium difficile* infection among older people in Tayside, Scotland, is associated with antibiotic exposure and care home residence: cohort study with nested case-control. J Antimicrob Chemoth, 3.

Mylotte, J.M., et al., 2012. Surveillance for *Clostridium difficile* infection in nursing homes. J Am Geriatr Soc, 61, 122-5.

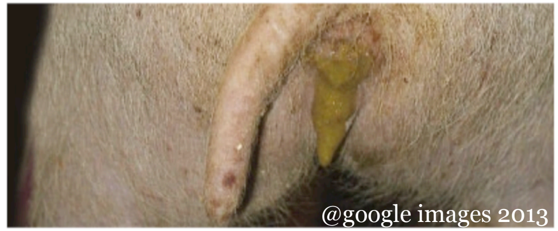
Pa Patient Saf Advis., 2010. *Clostridium difficile* infections in nursing homes. Pennsylvania Patient Safety Advisory, 18, 10-5.

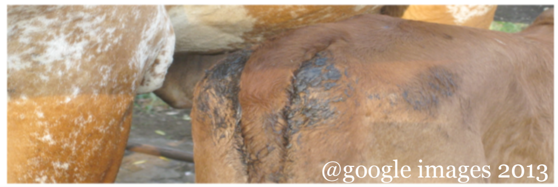
Simor, A.E., et al., 1993. Infection due to *Clostridium difficile* among elderly residents of a long-term-care facility. Clin Infect Dis, 17, 672-8.

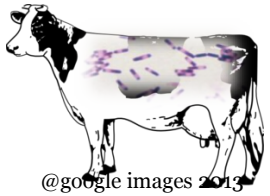
Ryan, J., et alF., 2010. Asymptomatic carriage of *Clostridium difficile* in an Irish continuing care institution for the elderly: prevalence and characteristics. Ir J Med Sci, 179, 245-50.

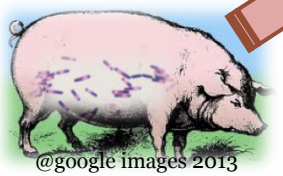
***Clostridium difficile* in animals and food**

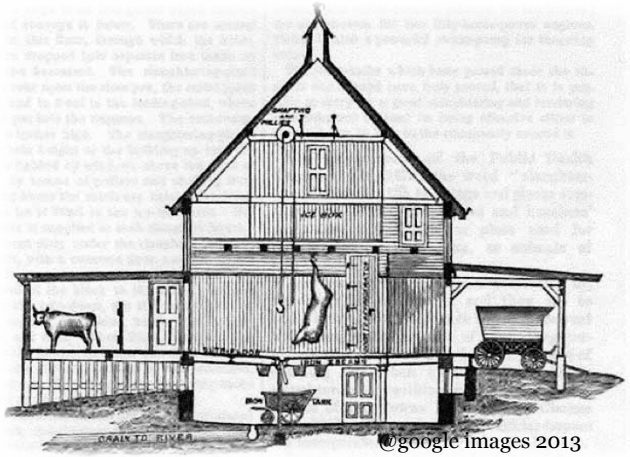
- In animals, *C. difficile* appears to be an important cause of enteric disease
- Asymptomatic carriage of *C. difficile* in animals has been also described
- *C. difficile* has been recently isolated from many types of meat products
- *C. difficile* meat isolates are correlated with the types implicated in human disease

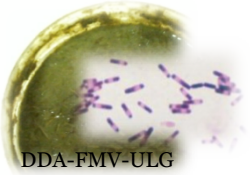


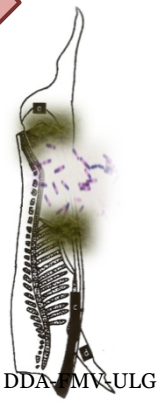












The possibility that *C. difficile* infection has a food or animal origin has recently been evoked due to the presence of *C. difficile* strains in food animals and meat. These strains are similar to those found in humans

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 animals at the slaughterhouse
- Evaluate the presence of *C. difficile* in retail meat
- 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**
  - Samples from 23 piglets were collected from 3 different breeding farms
  - Piglets were stimulated to make them defecate in individual tubes
  - The piglets don't have diarrhea and were still suckling

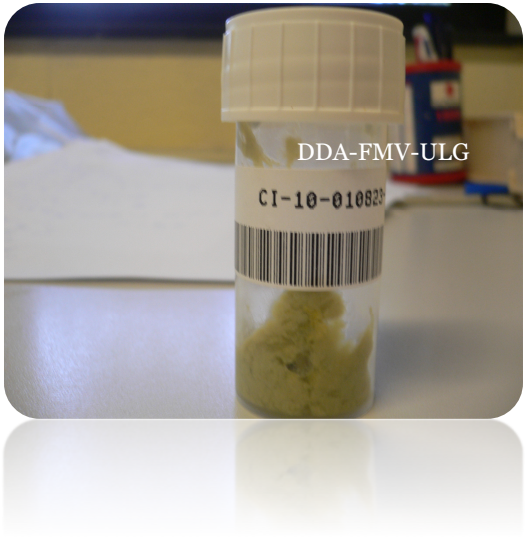
- **Calves faecal samples**
  - Faecal samples of 18 calves were collected from 5 different local farms
  - Calves were less than 3 months of age at the time of sampling

Study design

Slaughter animals

From January to July 2011

200 intestinal samples from cattle and pigs were collected over 9 different visits to a local slaughterhouse



All intestinal contents were collected from the slaughter line, directly from the large intestine in the viscera processing area

Study design

Intestinal contents, carcass samples and meat samples

From September to December 2011

A total of **100 intestinal samples** and **100 carcass samples** from **cattle** and **pigs** were collected **2 h** after slaughter in the chilling room.

Belgian Royal Decree of 20 August 2002

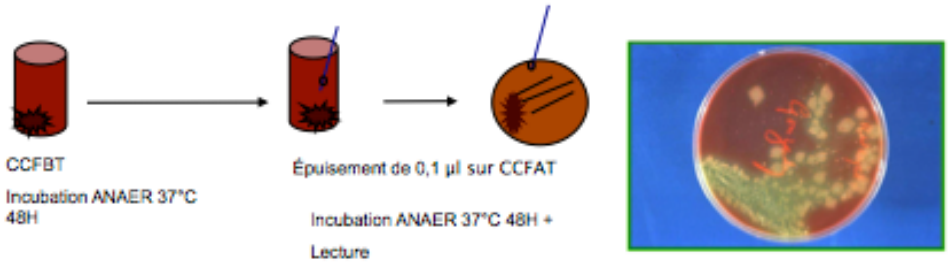
A total of 133 beef samples and 107 pork samples were collected

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, 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)

Results and 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 were 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.

Results and discussion: *C. difficile* on pigs and cattle carcasses

Prevalence 7%

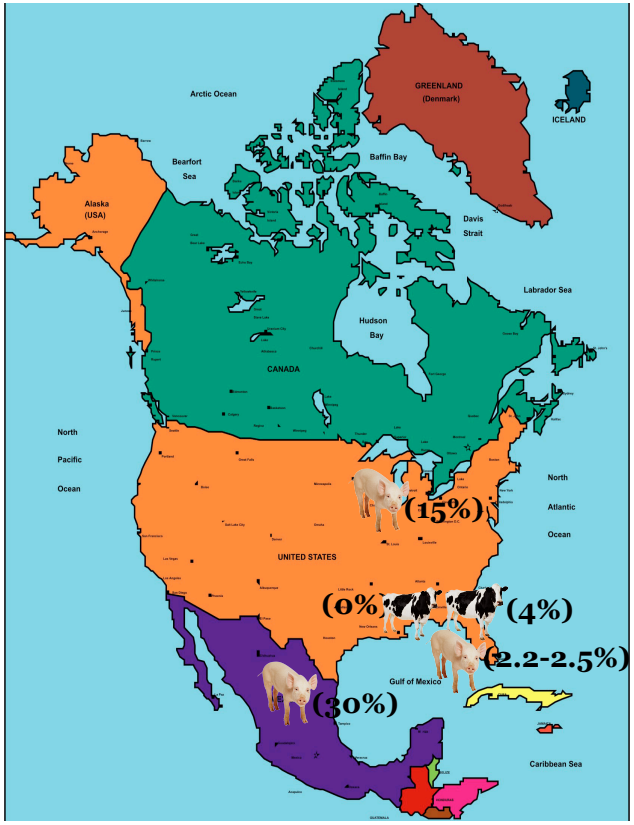
Main PCR-ribotypes 014/081/UCL36

Prevalence 7.9%

Great variety of types (UCL5a/UCL16u)

There are 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.

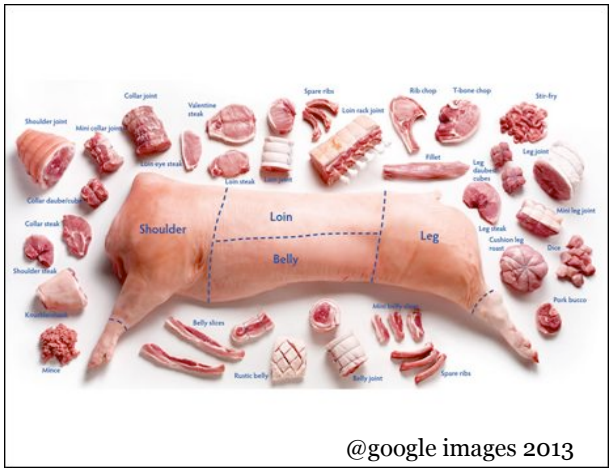
Hawkin,et al.,, 2012. Carriage and dissemination of *Clostridium difficile* and methicillin resistant *Staphylococcus aureus* in pork processing. Food Control, 31,433-37.

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.

Results and 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

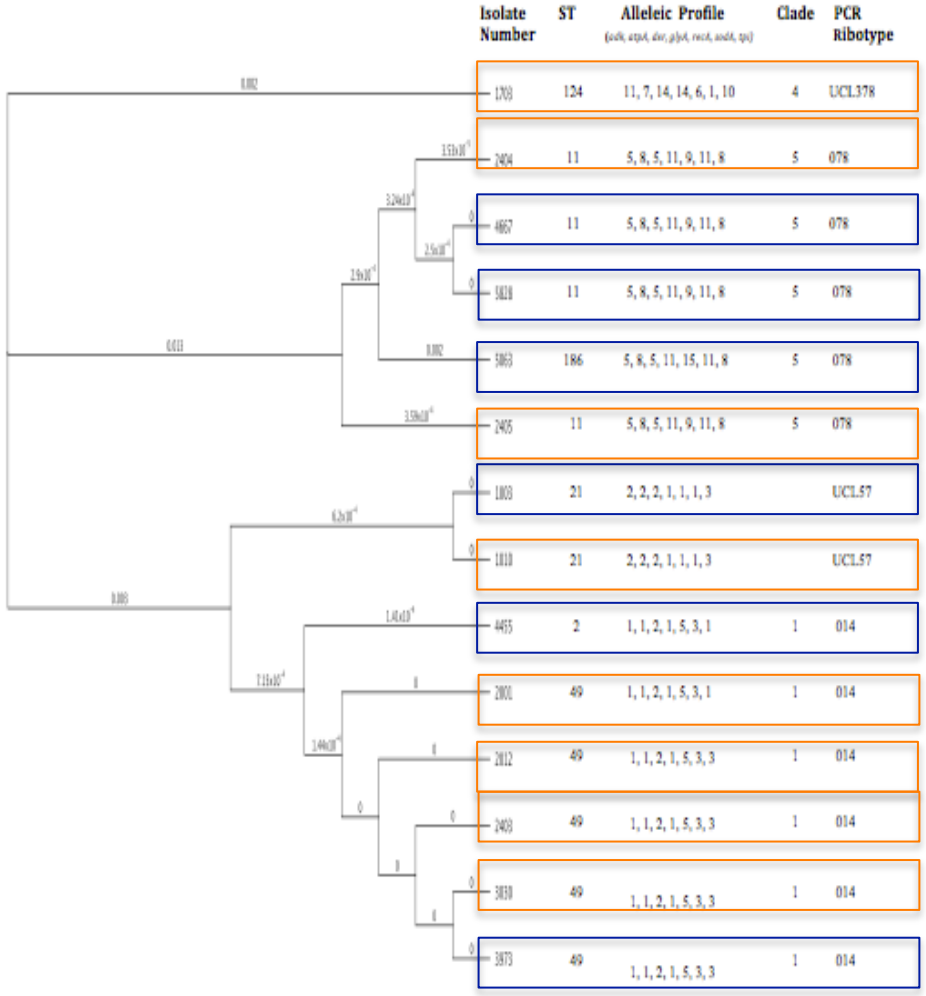
Hensgens et al., 2012. *Clostridium difficile* infection in the community: a zoonotic disease? Clin Microbiol Infec Dis 18, 635-45.

Discussion: Ribotypes distribution in Belgian hospitals

- In 2011 in Belgium, the most prevalent PCR ribotypes in hospitals were
  - **014*****, **002***, **027**, **078*****, **020**, **UCL46***, **UCL16l***, **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)



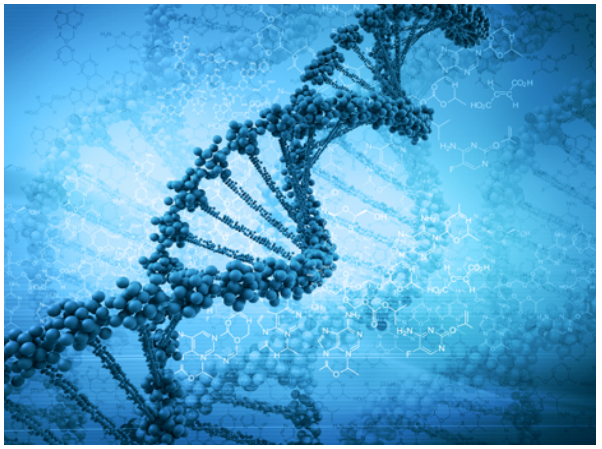
[1]Delmée, M., 2012. Epidemiology of *Clostridium difficile* in Belgium. NRC *Clostridium difficile*-Yersinia.

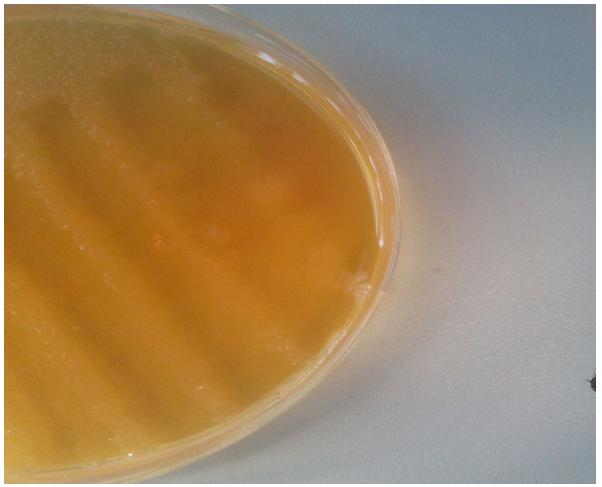
Conclusions

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

- PCR-ribotypes 014 and 078 were the most frequently identified. These ribotypes were also the most common isolates from patients in Belgium

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



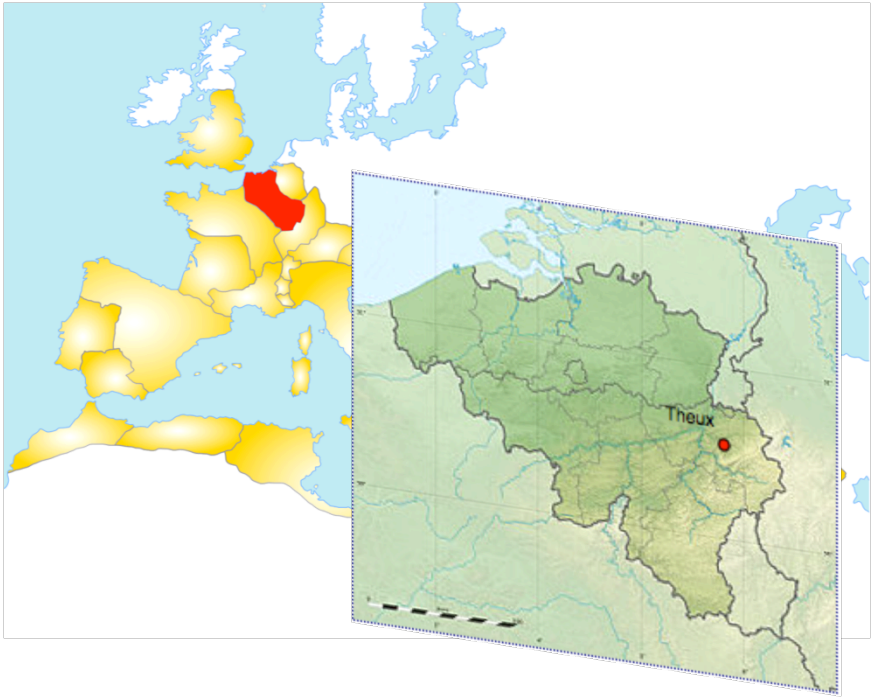


ASSOCIATION OF CLASSICAL MICROBIOLOGY AND TARGETED METAGENOMIC ANALYSIS TO EVALUATE THE PRESENCE OF *CLOSTRIDIUM DIFFICILE* IN A BELGIAN NURSING HOME

Objectives

- To evaluate and follow the prevalence of *C. difficile* among older people in a nursing home
- To establish a relationship between other intestinal bacterial populations and *C. difficile* colonization
- To evaluate the global evolutions of the total microflora and the relation with the *C. difficile* presence

Nursing home study



Capacity: 110 beds

- 34 nursing home
- 61 nursing home and long-term care
- 15 day centers

Employees

- 73

Study design

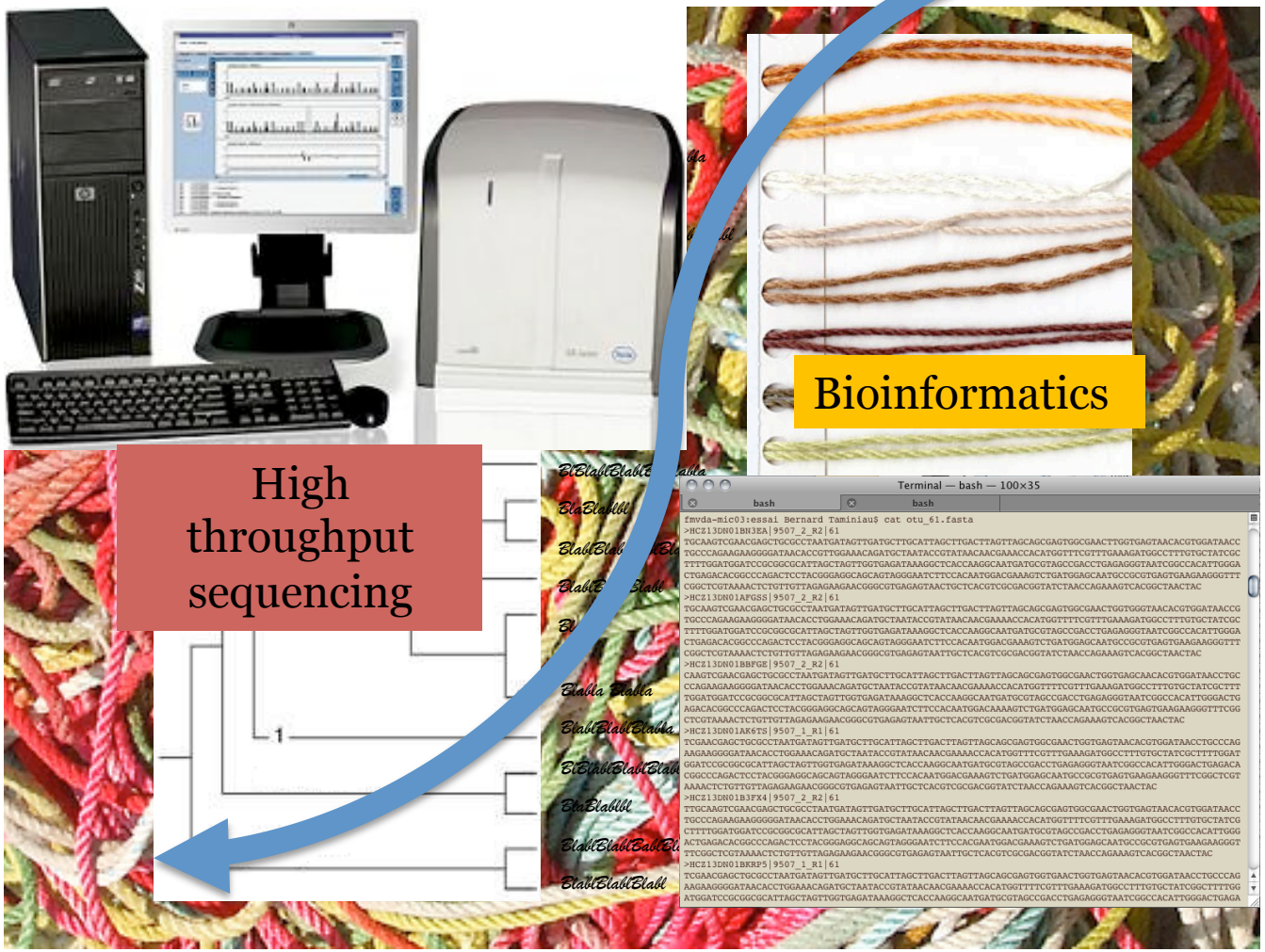
STOOL SAMPLES

From March to June 2013

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

- Two samples per person were collected: the first sample was cultivated and examined for *C. difficile* by classical microbiological methods and the second one was used to study the microbial biodiversity of the faeces content by amplicon sequencing coupled to microbial metagenomic analysis .

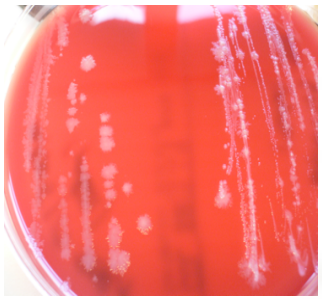
Metagenomics



Results: Prevalence of *C. difficile* in nursing home residents

C. difficile recovery:

- 7/23 (30.4%) residents were (at least one week) positive for *C. difficile*



- *C. difficile* was detected in 13/30 (43.3%) episodes of diarrhea and we found 25/47 (53.2%) samples positive but without diarrhea
- 4/13 (30.7%) residents positive for *C. difficile* had previously received an antibiotic therapy. 10/13 (77%) positive residents didn't get any antibiotic therapy

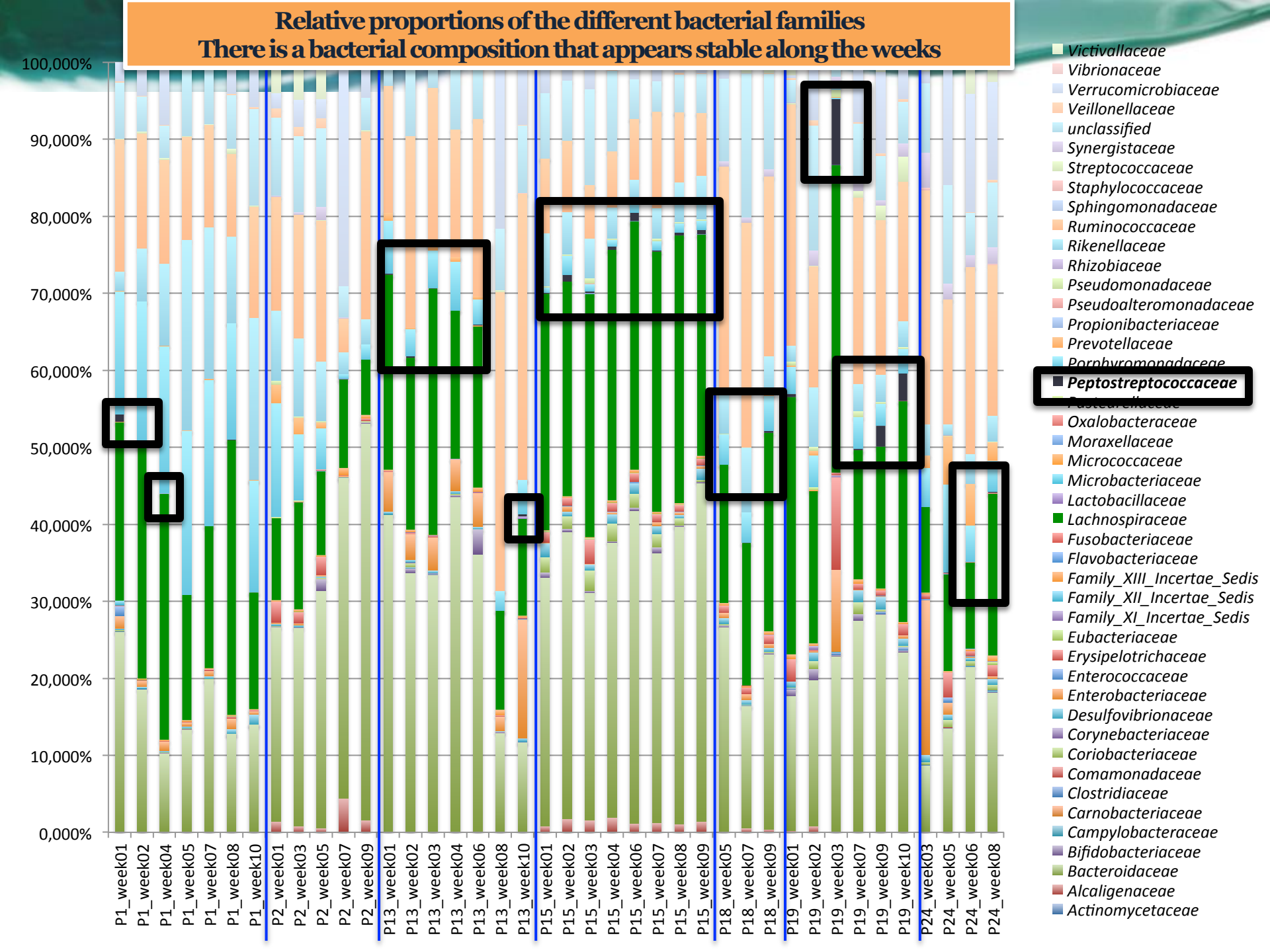
The results so far:

80 samples sequenced and analyzed: 6300 OTUs

Positive detection of *Clostridium difficile* :

C. difficile detection		
n	**Microbiology**	**Amplicon sequencing**
20	+	+
36	-	-
19	+	-
5	-	+

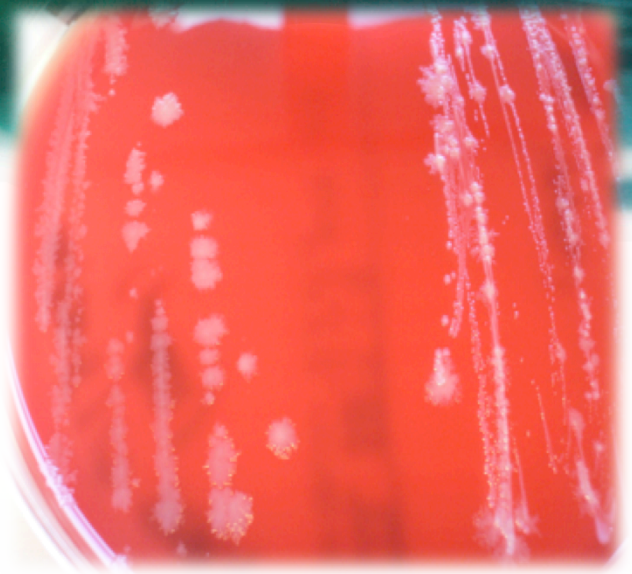
Relative proportions of the different bacterial families
There is a bacterial composition that appears stable along the weeks



The story so far

- *C. difficile* prevalence of 30.4% in a Belgian nursing home
- The most common PCR-ribotype identified was 027
- Residents have all their microbiota print
- Metagenomics analysis can't substitute targeted procotocols
- But It offers a global picture of the microbiota context:
  - With correlations
  - Identifications
  - Follow up

ACKNOWLEDGEMENTS



Public slaughterhouse of Liège

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