

*Clostridium difficile* infection: a zoonotic disease?



## *Clostridium difficile* infection in the community: a zoonotic disease?

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- ❖ Introduction
- ❖ Community-onset CDI and patient risk factors
- ❖ *C. difficile* in animals
- ❖ Source/transmission of *C. difficile*
- ❖ Preliminary results CO-CDI study in The Netherlands

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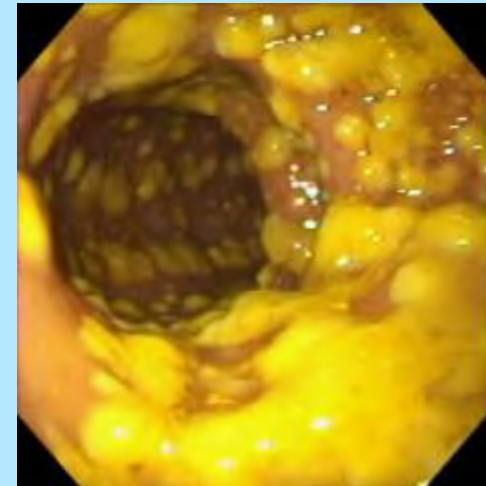
## *Clostridium difficile* infections (CDI)

*C. difficile* is ubiquitous in the environment and part of the normal gut flora in 2-4% of individuals

Spores play a major role in transmission of CD

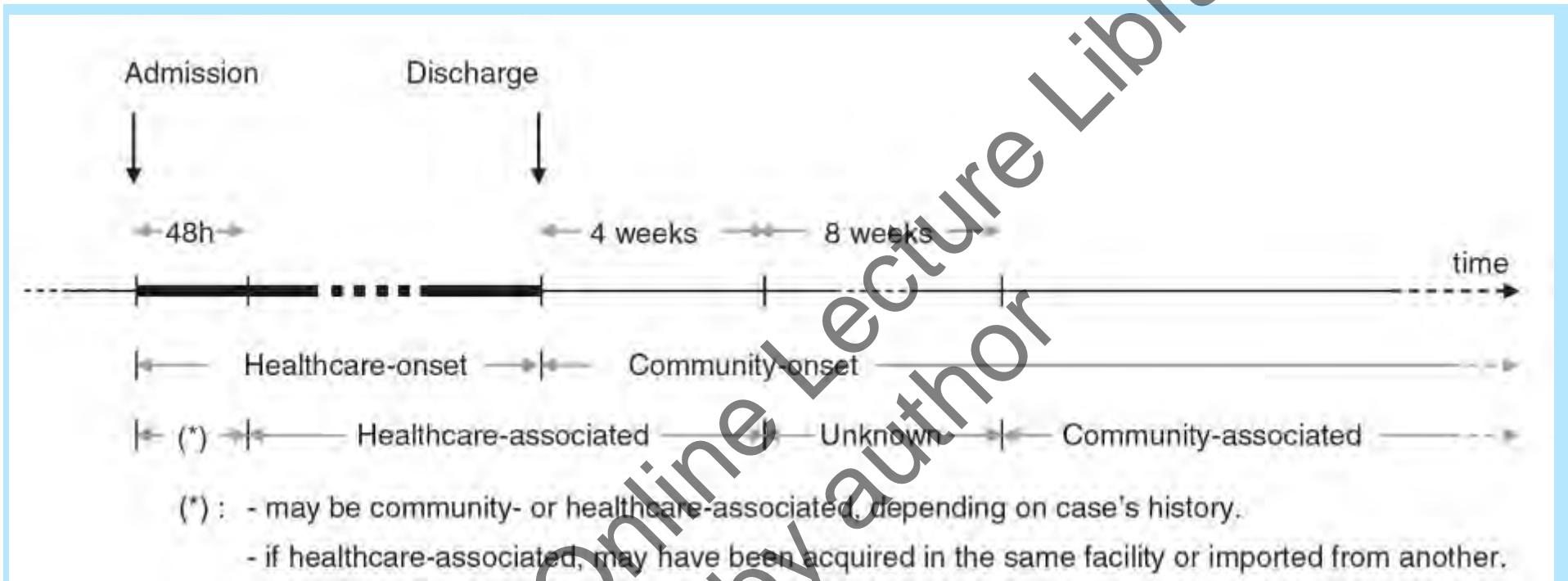
CDI: production of toxin A and/or B by vegetative cells in patients with predisposing conditions:

- Advanced age
- Increased disease severity
- Prior use of antibiotics
- (Previous) hospital admission



Barlett. *N Engl J Med.* 2002  
Dubberke. *Arch Intern Med.* 2007

## Definitions agreed upon by ESGCD, ECDC, and CDC



### Community acquired CDI;

- No hospitalization in previous 12 weeks before onset symptoms
- Onset outside a healthcare facility or within 48 hours after admission

# Increase of incidence and severity of HA-CDI

## Early 2003: Rise in incidence and severity of CDI

USA 200 / 10,000 admissions

>250,000 patients annually

NL 80 / 10,000 admissions

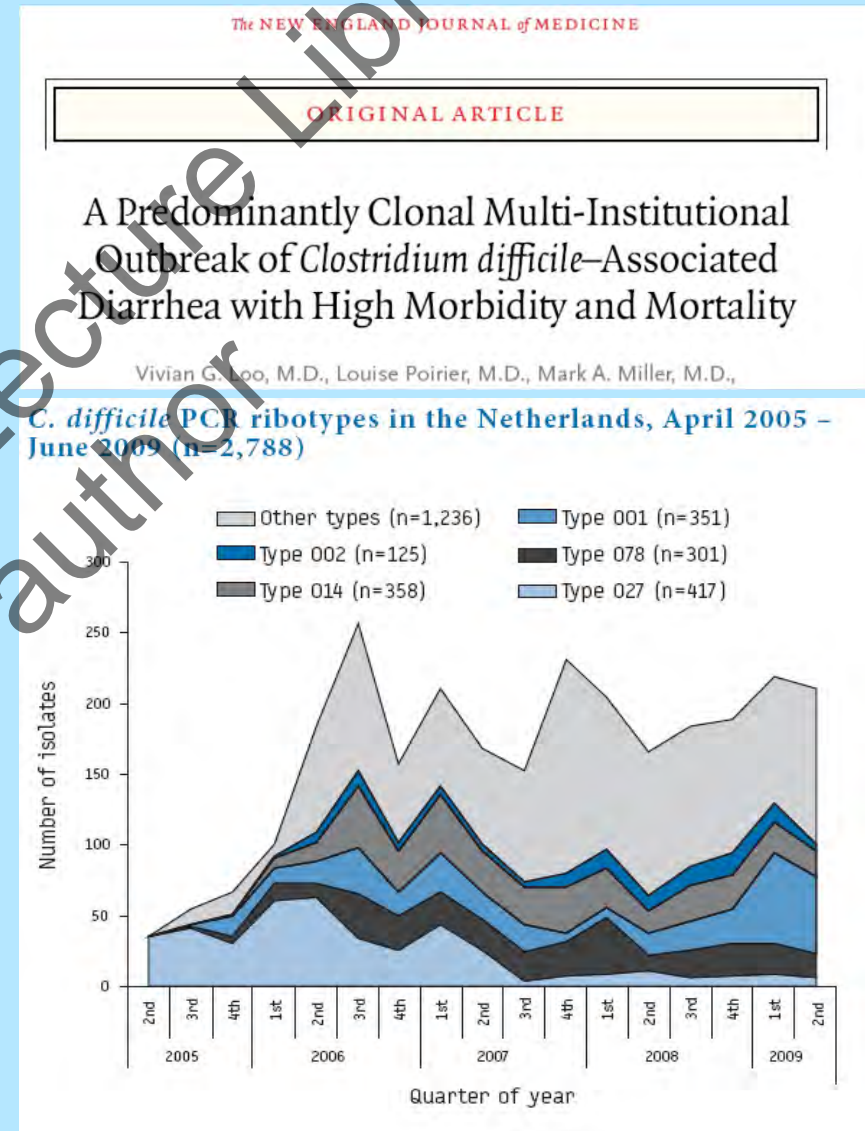
>2700 hospitalized CDI annually

### → Emergence of PCR ribotype 027

- High mortality and morbidity
- Frequent relapses

### Characteristics of the strain

<i>tcdA</i> and <i>tcdB</i>	Positive
<i>tcdC</i>	18 bp deletion
<i>tcdC</i>	Deletion at 117, frameshift
Binary toxin	Positive



## Incidence and severity of HA-CDI

### Emergence of *Clostridium difficile* Infection Due to a New Hypervirulent Strain, Polymerase Chain Reaction Ribotype 078

Abraham Goorhuis,<sup>1</sup> Dennis Bakker,<sup>1</sup> Jeroen Corver,<sup>1</sup> Sylvia B. Debast,<sup>2</sup> Celine Harmanus,<sup>1</sup> Daan W. Notermans,<sup>2</sup> Aldert A. Bergwerff,<sup>4</sup> Frido W. Dekker,<sup>5</sup> and Ed J. Kuijper<sup>1</sup>

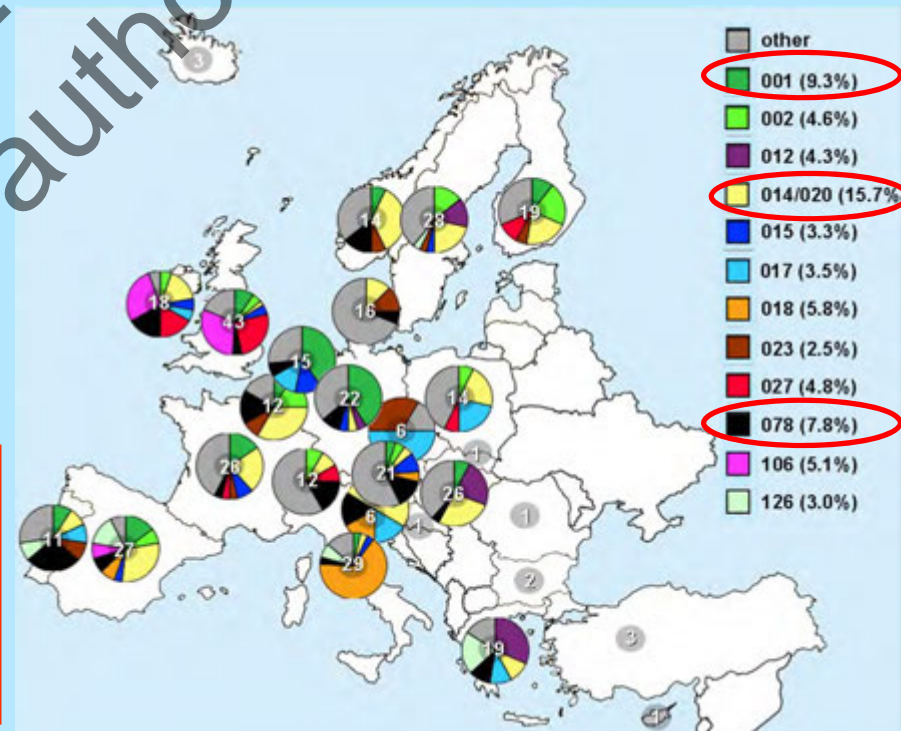
M. Bauer et al. Lancet 2011

#### *C. difficile* Type 078

- Similar severe CDI as type 027
- Younger population
- Associated with community acquired CDI

#### Characteristics of the strain

<i>tcdA</i> and <i>tcdB</i>	Positive
<i>tcdC</i>	39 bp deletion
<i>tcdC</i>	mutation at 184, stopcodon
Binary toxin	Positive



Risk factor	Proportion of patients with CDI (%)		
	Type 078	Type 027	Other types
Age, years			
0-64	47/145 (32.4)	59/270 (21.9)	418/1148 (36.4)
65-79	55/145 (37.9)	111/270 (41.1)	391/1148 (34.1)
• 80	43/145 (29.7)	100/270 (37.0) <sup>a</sup>	339/1148 (29.5)
Male sex	68/133 (51.1)	115/254 (45.3)	463/1052 (44.0)
Place of transmission			
Health care setting	41/57 (71.9)	112/120 (93.3) <sup>a</sup>	385/491 (78.4)
Community	10/57 (17.5)	8/120 (6.7) <sup>a</sup>	78/491 (15.9)
Indeterminate	6/57 (10.5)	0/120 (0.0) <sup>a</sup>	28/491 (5.7)
Outcome			
Severe diarrhea	21/54 (38.9) <sup>c</sup>	48/120 (40.0) <sup>d</sup>	134/476 (28.2)
Complicated course	5/52 (9.6)	22/124 (17.7) <sup>d</sup>	54/501 (10.8)
Recurrent infections	6/38 (15.8)	21/68 (30.9) <sup>c</sup>	62/333 (18.6)
Overall mortality	3/52 (5.8)	16/124 (12.9) <sup>d</sup>	35/501 (7.0)
Attributable mortality	2/52 (3.8)	5/124 (4.0) <sup>d</sup>	5/501 (1.0)
Contributable mortality	0/52 (0.0)	3/124 (2.4)	13/501 (2.6)

**Severe diarrhea;** bloody diarrhea and/or diarrhea with hypovolemia or hypoalbuminemia (albumin level, < 20 g/L), fever (temperature, >38.0°C) and leukocytosis (WBC count, >12x10<sup>9</sup> cells/L), and/or pseudomembranous colitis



## Is there also an increase of CDI in the community?

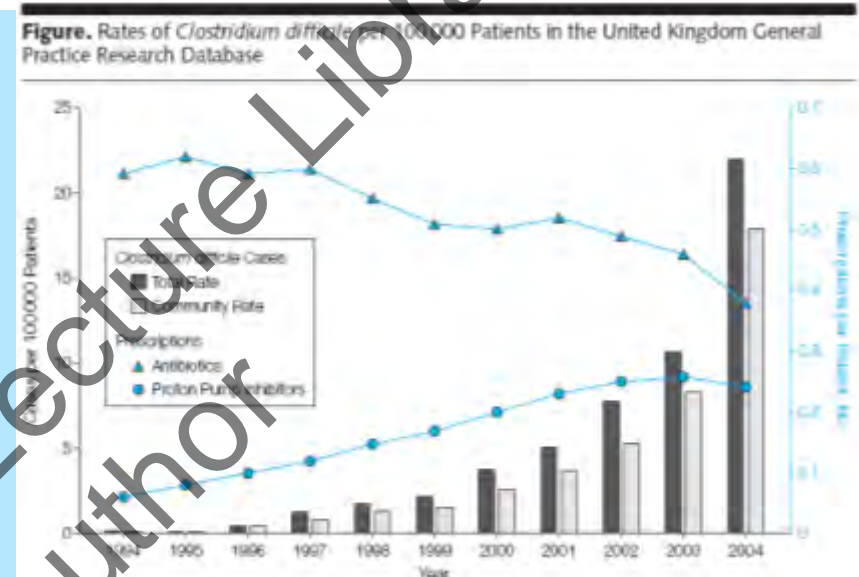
- ❖ Always an underestimation; only tested on request of physician and poor performances of EIA

- ❖ Most studies have methodological weaknesses

- ❖ summary of 12 studies on diarrhoeal patients to GP:

- GP: CDI incidence 7-25 / 100,000 ppy
- Community: 8-fold higher

Studies on patients with severe CA-CDI requiring hospitalization; approximately 5% of all in hospital diagnosed CDI



*Dial, JAMA, 2005*

## A case–control study of community-associated *Clostridium difficile* infection

M. H. Wilcox<sup>1,2\*</sup>, L. Mooney<sup>1</sup>, R. Bendall<sup>3</sup>, C. D. Settle<sup>4</sup> and W. N. Fawley<sup>1</sup>

Surveillance of community-derived faecal samples, case-control study

Of 2,000 randomly selected fecal samples, 2.1% positive for *C. difficile* cytotoxin. Type 001 most prevalent

### Risk factors for CO-CDI:

- Antibiotic use prior 4 weeks, hospitalization in prior 6 months  
→ 1/3 had no risk factor
- Contact with infants (<2 years),
- Animals were not investigated

**Conclusion:** Reliance on antibiotic history and age (65 years) will contribute to missed diagnoses of CA-CDI.

## Clinical and microbiological characteristics of community-onset *Clostridium difficile* infection in The Netherlands

M. P. Bauer<sup>1</sup>, D. Veenendaal<sup>2</sup>, L. Verhoef<sup>3</sup>, P. Bloembergen<sup>4</sup>, J. T. van Dissel<sup>1</sup> and E. J. Kuijper<sup>5</sup>

1) Department of Infectious Diseases, Leiden University Medical Centre, Leiden, 2) Public Health Laboratory Kennemerland, Haarlem, 3) SALTRO Artsenlaboratorium Utrecht, Utrecht, 4) Laboratory for Medical Microbiology and Infectious Diseases, Zwolle and 5) Department of Medical Microbiology, Leiden University Medical Centre, Leiden, The Netherlands

Bauer. CMI. 2009

- 3 months, 3 laboratories, all unformed stool from GPs tested for *C. difficile*. Of 2400 tested → 37 positive patients
- 13 different PCR ribotypes; unknown types followed by types 078, 172, 014.
- Age 1-92 years: 18% < 20 years
- 65% no admission previous year, 42% no use of antibiotics prior 6 months
  - 26% had no risk factor
- No patients had professional contact with farm animals
  - small study, no control group

## General practice

### Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance

Jeremy G Wheeler, Dinesh Sethi, John M Cowden, Patrick G Wall, Laura C Rodrigues,  
David S Tompkins, Michael J Hudson, Paul J Roderick on behalf of the Infectious Intestinal Disease  
Study Executive

*Wheeler, BMJ, 1999*

- Population based , community cohort incidence study
- 1993-1997; 9776 patients, randomly selected from list of GP and prospectively questioned on diarrhoea during period of 6 months
- Infectious diarrhoea : 1 per 5 individuals of whom 1 per 6 visited GP
- Diarrhoea occurred in 781 cases; and 6 (0.7%) had CDI (CCA)
- Incidence is 160 per 100,000 persons per year.

**Table 1** Incidence of infectious intestinal disease in community and reported to general practice by organism

	Community		General practice		No of community cases/ GP case (95% CI)
	No of cases*	Rate/1000 person years (95% CI)	No of cases	Rate/1000 person years (95% CI)	
<b>Bacteria:</b>					
<i>Aeromonas</i> spp	46	12.4 (9.4 to 16.7)	165	1.88 (1.48 to 2.37)	6.7 (4.9 to 9.1)
<i>Bacillus</i> spp (>10 <sup>4</sup> /g)	0	0	1	0.05 (0.01 to 0.15)	—
<i>Campylobacter</i> spp	32	8.7 (6.1 to 12.3)	254	2.14 (3.34 to 5.13)	2.1 (1.5 to 3.0)
<i>Clostridium difficile</i> cytotoxin	6	1.6 (0.7 to 3.6)	17	0.20 (0.12 to 0.31)	8.0 (3.4 to 19.3)
<i>Clostridium perfringens</i> enterotoxin	9	2.4 (1.0 to 4.7)	114	1.30 (1.04 to 1.68)	1.9 (0.97 to 3.7)
<i>E coli</i> O157	0	0	3	0.03 (0.01 to 0.11)	—
<b><i>E coli</i> DNA probes:</b>					
Attaching and effacing	20	5.4 (3.5 to 8.4)	119	1.32 (1.10 to 1.62)	4.1 (2.6 to 6.5)
Diffusely adherent	23	6.2 (4.2 to 9.4)	103	1.18 (0.92 to 1.52)	5.3 (3.4 to 8.2)
Enterocytotoxic	18	4.9 (3.1 to 7.8)	141	1.62 (1.30 to 2.03)	3.0 (1.9 to 4.9)
Enteroinvasive	0	0	0	0	—
Enteropathogenic	1	0.27 (0.04 to 1.9)	4	0.05 (0.01 to 0.15)	5.4 (0.8 to 55.7)
Enterotoxigenic	10	2.7 (1.5 to 5.0)	52	0.59 (0.44 to 0.81)	4.6 (2.4 to 8.9)
Verocytotoxigenic (non-O157)	3	0.82 (0.26 to 2.5)	6	0.06 (0.02 to 0.17)	13.4 (3.6 to 49.6)
<i>Salmonella</i> spp	8	2.2 (1.1 to 4.3)	146	1.57 (1.19 to 2.06)	1.4 (0.7 to 2.8)
<i>Shigella</i> spp	1	0.27 (0.04 to 1.9)	23	0.27 (0.16 to 0.47)	1.0 (0.13 to 7.3)
<i>Staphylococcus aureus</i> (>10 <sup>6</sup> /g)	1	0.27 (0.04 to 1.9)	10	0.11 (0.05 to 0.23)	2.5 (0.33 to 19.0)
<i>Vibrio</i> spp	0	0	1	0.01 (0.001 to 0.05)	—
<i>Yersinia</i> spp	25	6.8 (4.6 to 10.0)	51	0.58 (0.42 to 0.88)	11.7 (7.5 to 18.3)

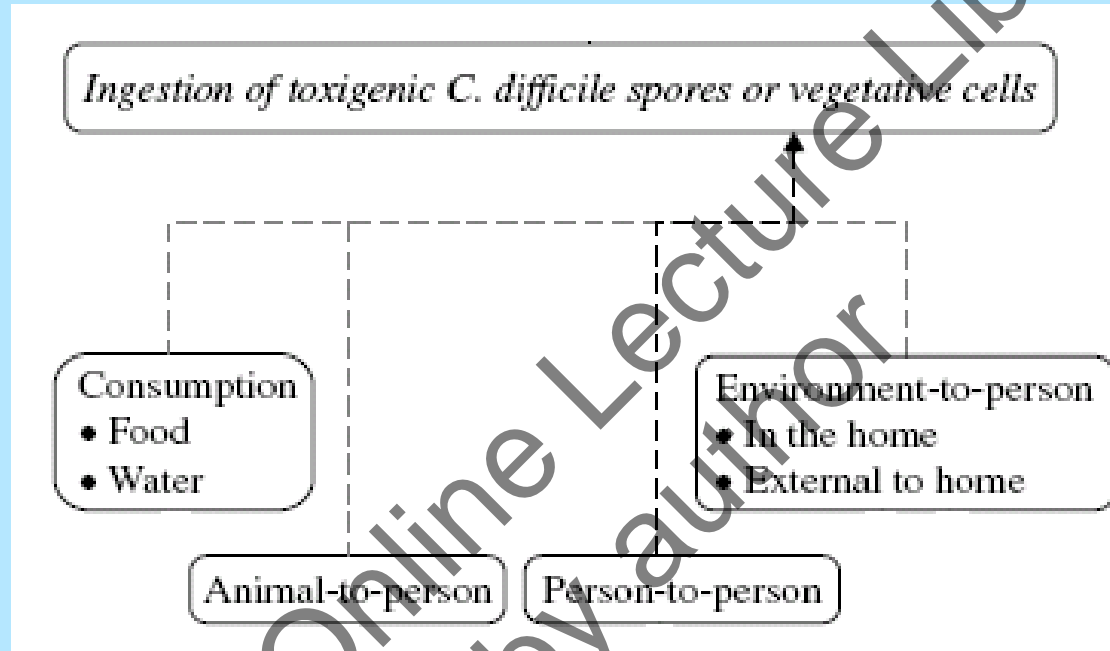
## Risk factors for CDI with onset in the community

- **Not a classical profile:** mean age frequent <60 yrs
- 11 studies: 15-40% no antibiotic use or recent HCF admission

Author	Study period	Setting	N	Prior antibiotic usage
Fellmeth et al.	2008-2009	GP, UK	54	32% in 4 weeks
Dial et al.	1994-2004	GP, UK		37% in 3 months
Wilcox et al.	1999	GP, UK	42	52% in 1 month
Bauer et al.	2008	GP, the Netherlands	37	58% in 6 months
Hirschhorn et al.	1988-1990	GP/hospital, USA	51	65% in 42 days
MMWR	2006	GP/hospital, USA	241	68% in 3 months
Riley et al.	1988	GP, Australia	16	69% in 3 months
Kuntz et al.	2004-2007	GP/hospital, USA	304	73% in 6 months
Khanna et al.	1991-2005	GP/hospital, USA		78% in 3 months
Riley et al.	< 1994	GP, Australia	13	85% in 4 weeks
Karlstrom et al.	1995	GP/hospital, Sweden	529	88% in 6 weeks

- **Susceptibility and acquisition of *CDI* is not well understood**
- Proton pump inhibitors postulated to increase vulnerability to *C. difficile*

## Transmission of *C. difficile*



Otten, *Epidemiol Infect*, 2010

Animals as source for humans by direct, indirect contact, the environment or the food?

## *Clostridium difficile* in animals

- Numerous animal species, including fish
- Pets, calves, horses, poultry and pigs most frequently investigated

*Rupnik, CMI, 2007*





## **Pets**

- 21% of dogs and 30% of cats carriers without signs of disease Borriello, 1983
- Some studies found significant relation between *C. difficile* and enteritis Riley, 1991
- Keessen, Review in Vet. Microbiol, 2010

## **Food producing animals, 1996**

Al Saif, 1996

- 40 farms, >100 animals (cattle, sheep, poultry, pigs and horses) *C. difficile* hardly isolated. Highest prevalence in poultry (1.6%), not isolated from the pigs or cattle

**21st century:** epidemiology has changed

## *Clostridium difficile* in animals



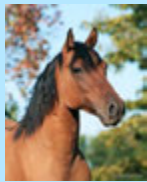
### **Pigs:**

- Kochs postulate confirmed in 2 studies
- No association between disease and presence of *C. difficile* found in Spain

**However:** pathogenesis and role of toxins still need to be established



**Calves:** no established relation of *C. difficile* with disease



**Horses:** low prevalence (0-1.2%) in horses without diarrhoea, high prevalence (6-40%) in foals and adult horses with diarrhoea



**Poultry:** up to 62% of 61 chicken feces (non-diarrhoeal) samples positive, age dependent

## *Clostridium difficile* PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans

Environmental Microbiology (2008)

Sylvia B. Debast,<sup>1</sup> Leo A. M. G. van Leengoed,<sup>2</sup>  
Abraham Goorhuis,<sup>3</sup> Celine Harmanus,<sup>3</sup>  
Ed J. Kuijper<sup>3</sup> and Aldert A. Bergwerff<sup>1\*</sup>

- 2 farms with outbreaks of diarrhoea in piglets (1 year)
- High morbidity (80%), low mortality (12%), growth rates were affected
- Periparturient medication of sows with trimethoprim-sulfadiazin, vaccination and use of amoxicilline
- Yellow to orange watery diarrhoea
- Exsudative fibrino-haemorrhagic colitis of colon, but no necrotic lesions in mucosa of small intestine (*C. perfringens*)
- Cultures for *C. perfringens* negative, no *Isospora suis* or rotavirus



## *Clostridium difficile* PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans

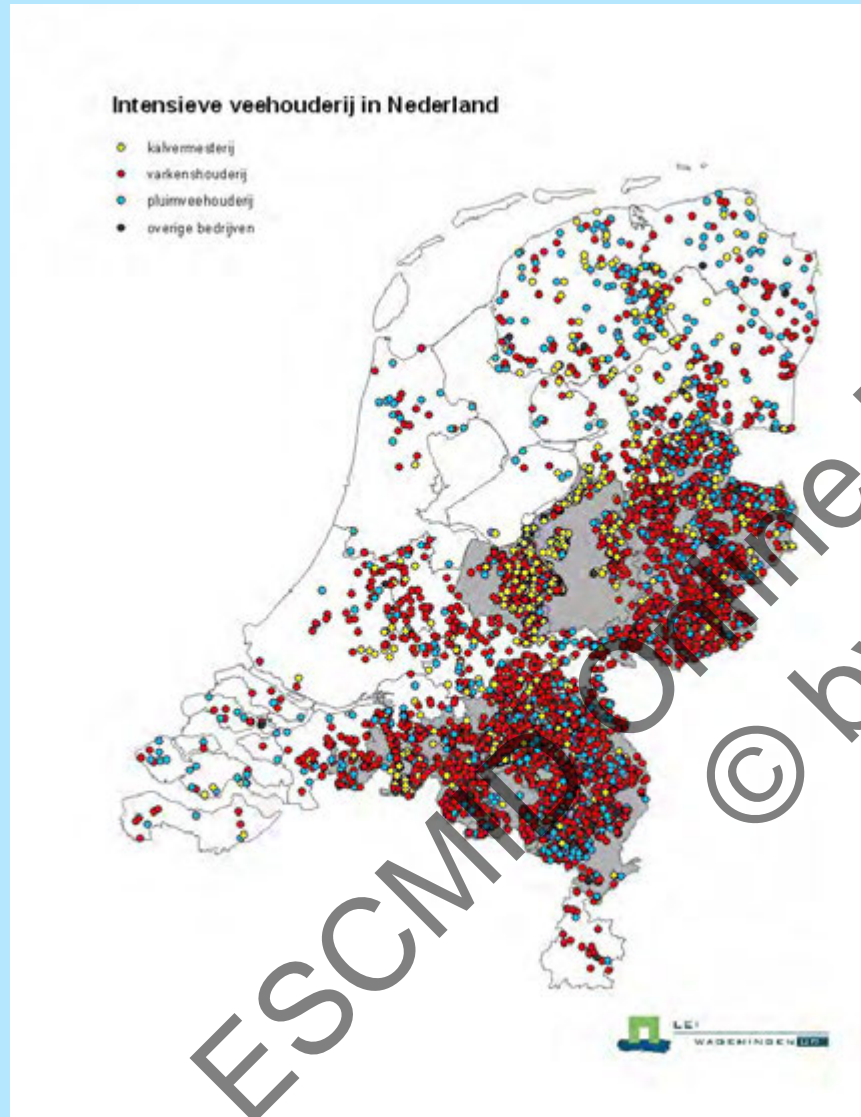
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Sylvia B. Debast,<sup>1</sup> Leo A. M. G. van Leengoed,<sup>2</sup>  
Abraham Goorhuis,<sup>3</sup> Celine Harmanus,<sup>3</sup>  
Ed J. Kuijper<sup>3</sup> and Aldert A. Bergwerff<sup>1\*</sup>

- Feces samples (n=48) from 1-4 day-old piglets **with diarrhoea**  
→ 31 *C. difficile* isolates were obtained.
- 272 **healthy** weaned piglets on 7 farms  
→ no *C. difficile*.
- None of the 20 mother sows contained *C. difficile*

**Conclusion:** *C. difficile* is associated with disease in piglets

- None of the farm-workers and none of the family members of the farm-owners had CDI



30 of 32 pig farms were positive for presence of *C. difficile*

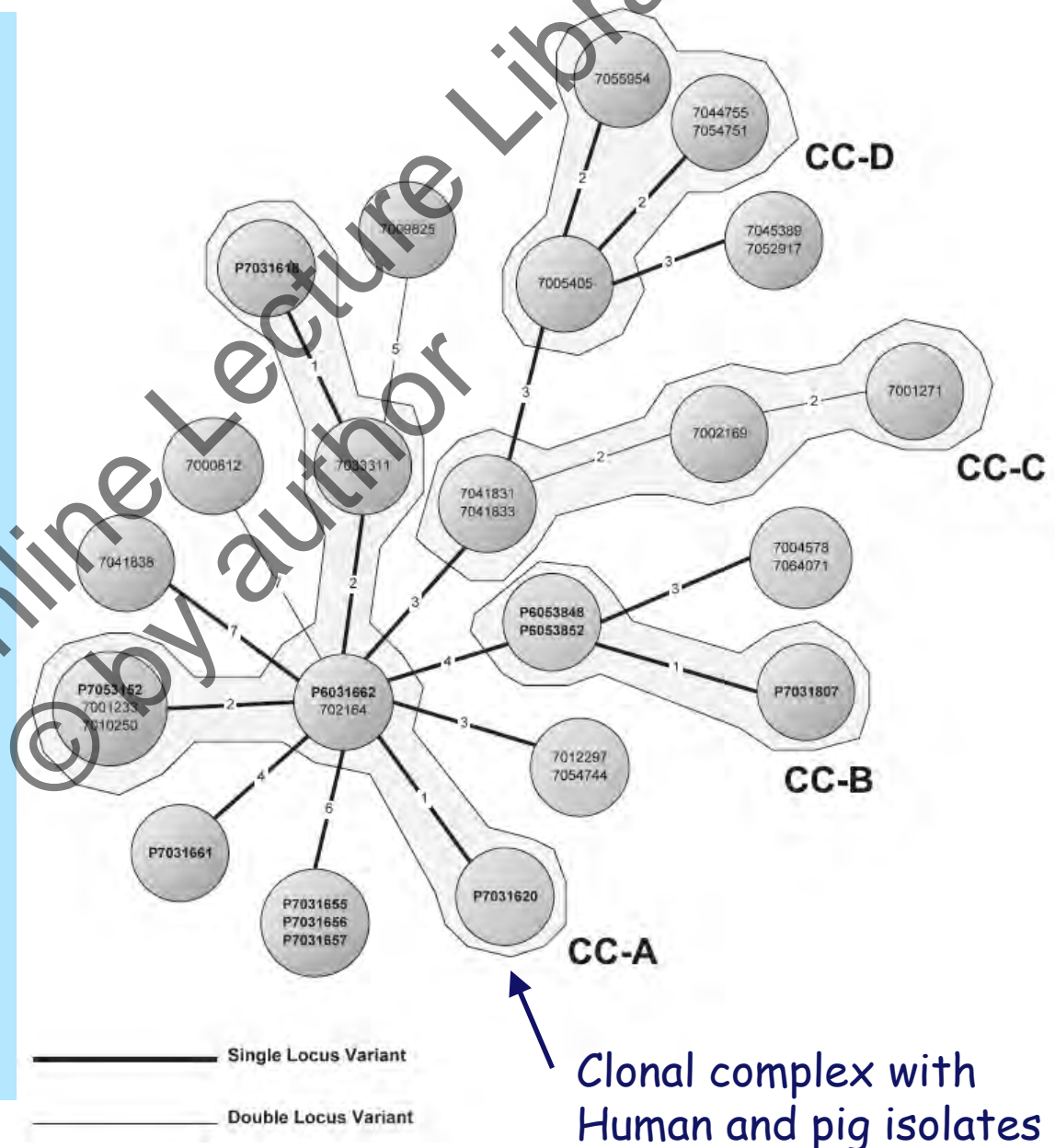
MLVA type 078

- ❖ MLVA of 31 *C. difficile* type 078 isolates:
- ❖ 21 human isolates and 11 porcine isolates.

All genetically related

4 clonal complexes

**CC-A:** both human and porcine isolates  
 → 2 porcine isolates were 100% homologous with 1 and 2 human isolates



## *C. difficile* in meat



### Food and Consumer Product Safety Authority (Vlaamse Voedsel en Waren Autoriteit)

- ❖ Independent agency in the Ministry of Agriculture, Nature and Food Quality (LNV)
- ❖ Quality control of consumable meat samples from groceries
- First months of 2009 *C. difficile* in food investigated by enrichment broth
- Of 500 meat samples (calves, pigs, sheep, turkey, chicken), 4 positive for toxinogenic *C. difficile*: Types 001, 003 (2) and 087.
- All positive samples from chicken

**Conclusion:** low prevalence with “human” types.

## *C. difficile* in meat

**Remarkably:** Europe reports low prevalences compared USA/Canada

- High isolation frequencies, but quantitative studies show low level contamination (<100 CFU in chicken and 20-240 spores/g in retail beef and pork)
- Majority toxinogenic
- Clear overlap in types found in humans

	Country	N pos / N	(%)	most frequent type	
<b>Retail beef and veal</b>					
North America	Canada	1/7	14%	027	
	Canada	11/53	21%	027	
	Canada	10/149	7%	027	
	USA	3/65	5%	078	
	USA	14/33	42%	078	
Europe	Canada	14/115	12%	078	
	USA	4/50	8%	-	
	Sweden	2/32	2%	-	
	France	2/105	2%	012	
	Austria	0/30	0%	053	
	Austria	3/70	4%	-	
Retail pork	Netherlands	0/164	0%	-	
	Switzerland	0/46	0%	-	
	North America	USA	19/46	41%	078
		Canada	14/115	12%	078
Canada		7/393	2%	027	
USA		23/243	10%	078	
Europe	France	0/59	0%	-	
	Austria	0/27	0%	-	
	Netherlands	0/63	0%	-	
<b>Poultry products</b>					
North America	USA	4/9	44%	078	
	Canada	26/203	13%	078	
	USA	4/32	13%	078	
Europe	Austria	0/6	0%	-	
	Netherlands	7/257	3%	003	



## *C. difficile* in other food products

- Meat received most attention, limited information on other food products

<b>Country</b>	<b>Product type</b>	<b>N pos / N</b>	<b>(%)</b>	<b>most frequent type</b>	<b>Reference</b>
Canada	Vegetables, divers	5/111	5%	078	Metcalf et al. (2010)
Canada	Seafood/fish	5/119	5%	078	Metcalf et al. (2011)
Canada	Dog and cat feed	1/25	4%	-	Weese et al. (2005)
UK	Raw vegetables	7/300	2%	-	al Saif and Brazier
	Fish gut contents	0/107	0%	-	
UK	Ready-to-eat salads	3/40	8%	017	Bakri et al. (2010)
Austria	Raw milk	0/50	0%	-	Jöbstl et al. (2010)

- Spores of *C. difficile* can be recovered from practically any environmental site?
- Wales 1996; 21% of soil samples, 88% of river samples, 50% of sea, lake and swimming pools, 5.5% of tap water.
- 1-5 CFU/ml ( al Saif et al, J Med Microbiol 1996;45:133-137)
- Slovenia; 61% of 69 river isolates positive (Zidaric et al, Anaerobe 2010; 16: 371-5)
- In total, 34 different PCR ribotypes of which 001 was found in more than 50%.

# Aerial dissemination of *Clostridium difficile* on a pig farm and its environment

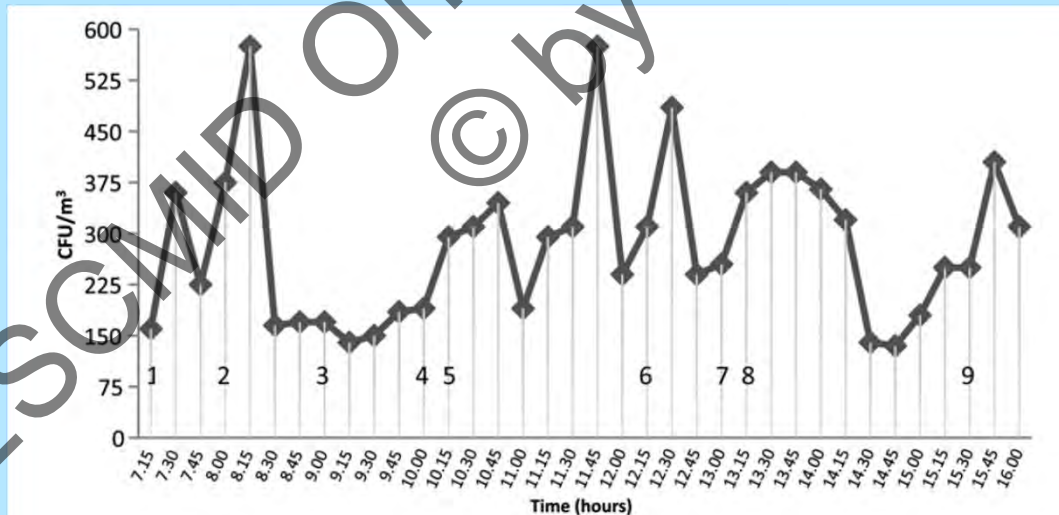
E.C. Keessen<sup>a</sup>, C.J. Donswijk<sup>a</sup>, S.P. Hol<sup>a</sup>, C. Hermanus<sup>b</sup>, E.J. Kuijper<sup>b</sup>, L.J.A. Lipman<sup>a,\*</sup>

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Environmental Research 111 (2011) 1027–1032

- ❖ To investigate the occurrence of *C. difficile* in and nearby a pig farm with a high prevalence of *C. difficile* Type 078
- ❖ Airborne colony counts peaked on the moments shortly after or during personnel activity in the pens.
- ❖ A decrease in airborne colony counts was observed parallel to aging of the piglets
- ❖ Airborne *C. difficile* was detected up to 20 m distant from the farm.



## High colonization rate of farmers at pig farms with emerging *Clostridium difficile* PCR ribotype 078

L. Keessen, E. Kuijper, L. Lipman

- ❖ 32 pig farms, 30 farms with Type 078 and 1 farm 045
- ❖ Of 56 farmers and employees, **14 (25%)** were positive for fecal carriage of *C. difficile*
- ❖ Of 32 partners, 4 (13%) were positive (OR daily contact with pigs >2)
- ❖ Of 41 children, none was positive
- ❖ Typing revealed all Type 078, except for 1 farm (type 045)

## Prospective case-control study of CDI in the community

*M. Hensgens, O. Dekkers, E.J Kuijper .*

Study of CDI among patients with diarrhoea visiting the general practitioner

### Aims:

- ❖ Determine risk factors for CA-CDI
- ❖ Determine risk factors for CDI due to type 078
  - ❖ Contact with cattle (especially pigs)
  - ❖ Foodborne transmission
  - ❖ Environmental factors



### Secondary aims

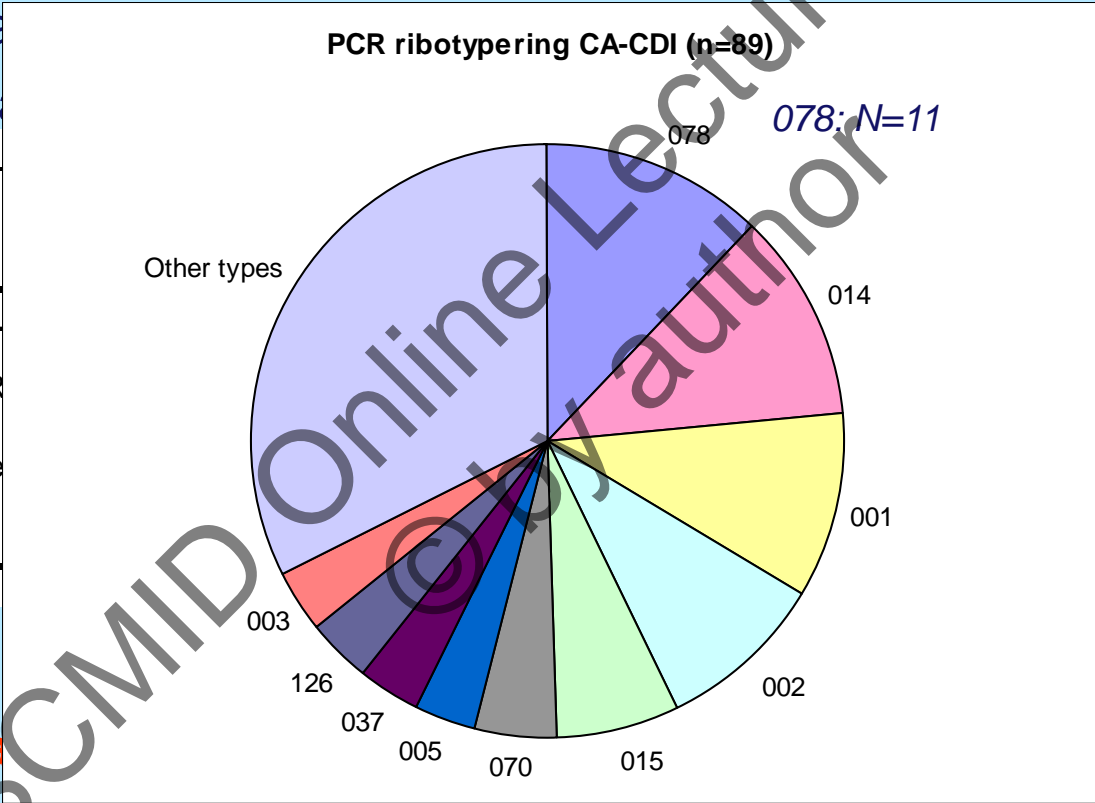
- ❖ Epidemiology of ribotypes in CA-CDI
- ❖ Mortality and morbidity of CA-CDI after 6 months

## Preliminary results

10.000 patients with diarrhoea who presented to their GP were tested

- ❖ In total 175 cases detected
- ❖ 130 patients
- ❖ First hospital

Characteristics/Risk
Sex (% male)
Age in yrs (median, IQR)
Admission to healthcare
Any diseases
Use of any antibiotics



→ contact

patients with 078?

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## Conclusions

1. The epidemiology and knowledge of human and animal CDI has changed in the recent years
2. Direct transmission of *C. difficile* from animals or foods to humans has not been proven, though similar PCR ribotypes have been found.
3. No outbreaks reported in the country
4. Emerging type 078 has zoonotic potential

**Thank you  
for  
your attention**



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Birgit van Benthem (RIVM)

Miriam Koene, Dik Mevius (CVI)

Liny Keessen (Utrecht)

Len Lipman (Utrecht)



