



Institute of Public
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Medical Faculty
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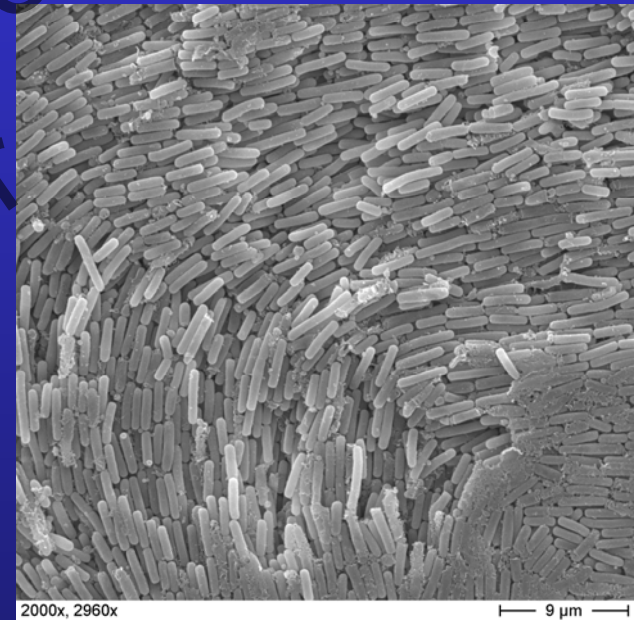
On food, animals and *Clostridium difficile*

Maja Rupnik

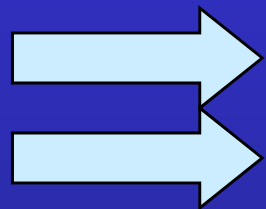
Institute of Public Health Maribor and
Medical faculty, University of Maribor, Maribor, Slovenia

Clostridium difficile

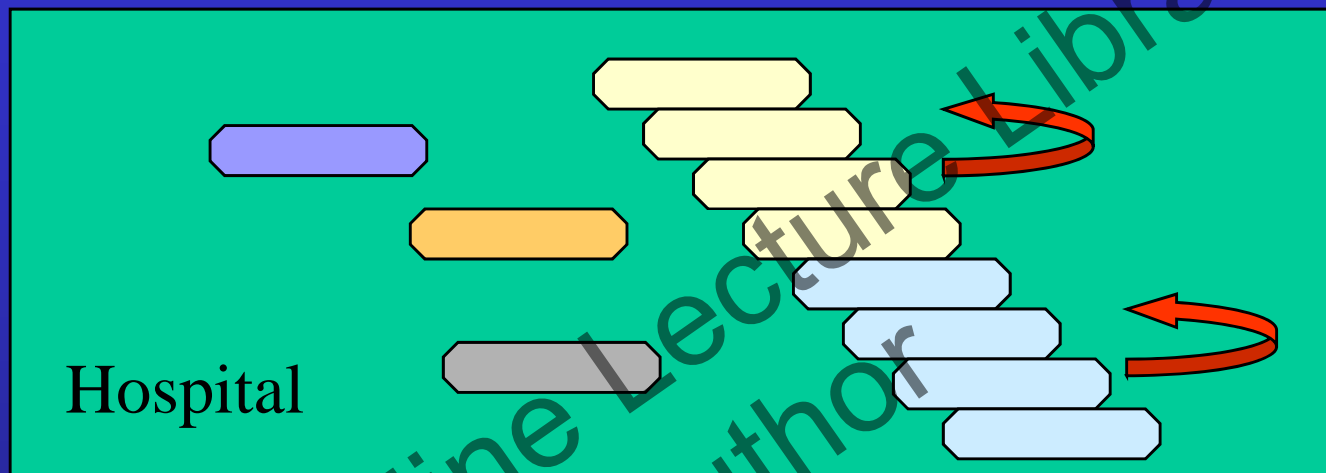
- anaerobic, sporogenic, Gram positive bacterium
- toxin mediated intestinal disease (diarrhoea, pseudomembranous colitis)
- disturbed normal intestinal flora
- nontoxigenic strains
toxigenic strains
- large clostridial toxins (TcdA, TcdB)
binary toxin (CDT)



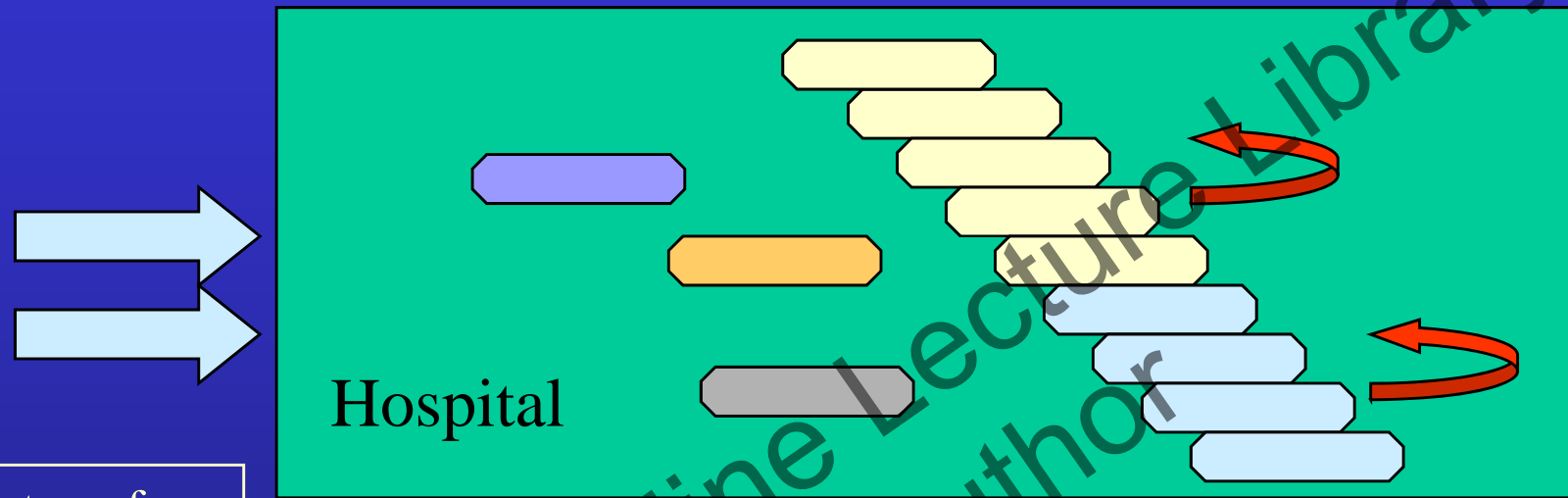
Nosocomial vs. community-acquired



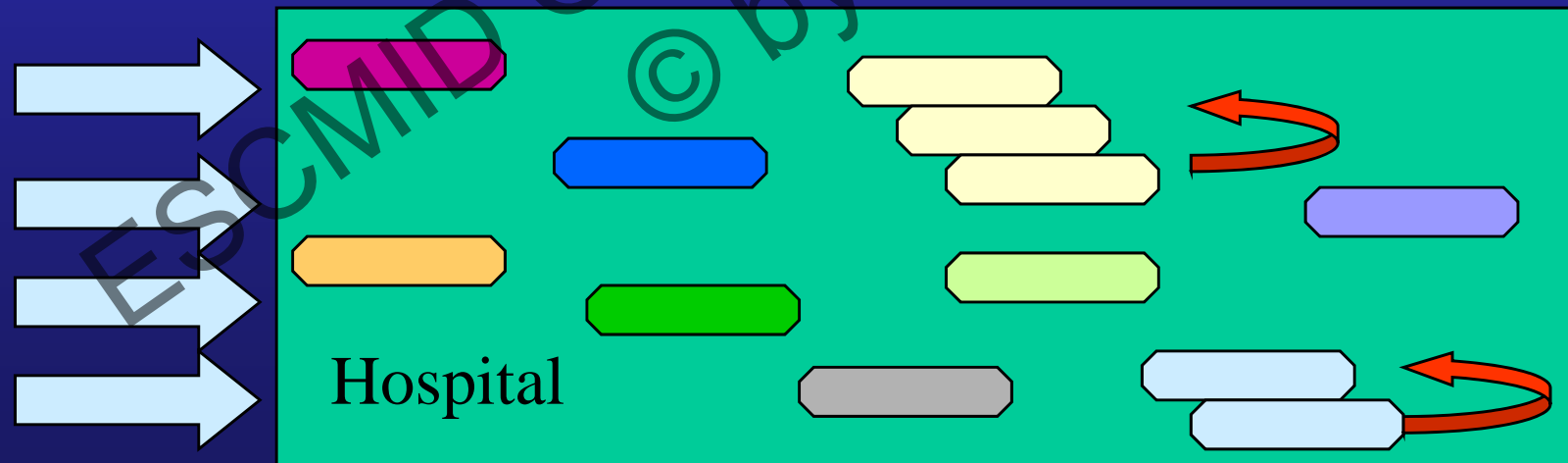
introduction of
new types RARE



Nosocomial vs. community-acquired

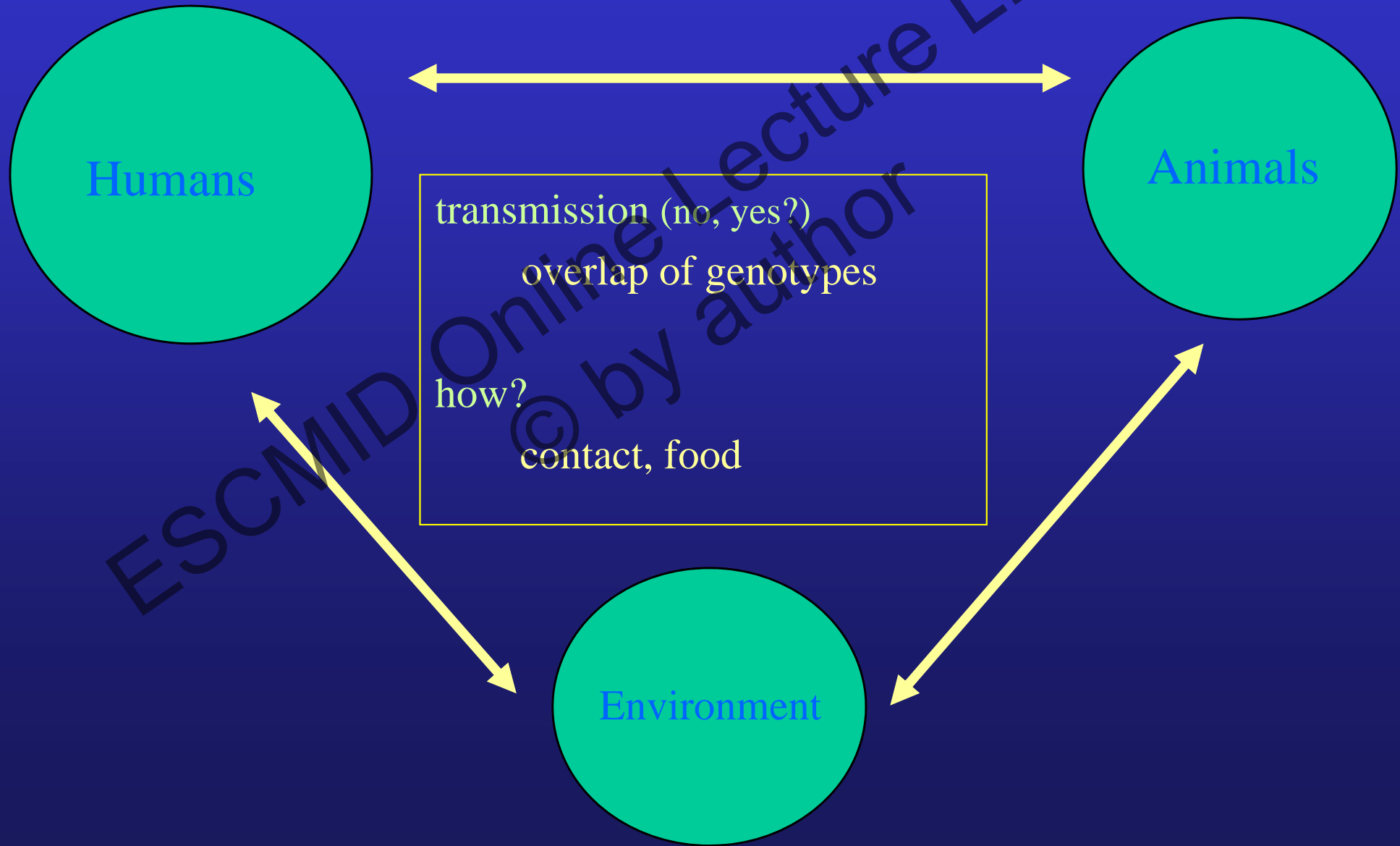


introduction of
new types RARE



introduction of new
types FREQUENT

Clostridium difficile – other reservoirs?



C. difficile in animals

- *C. difficile* is described in several animal species
camels, seals, deer, elephant, tiger
laboratory rodents (hamster, guinea pigs, rabbits, mice)
cats, dogs, horses, pigs, calves, poultry
- disturbed normal gut flora (!)
antibiotics
young age
diet (inhibiting growth of intestinal microorganisms; Bojesen et al., Vet. Microbiol., 2006)
- diseased or healthy animals (multiplying hosts)
no clear link between presence of *C. difficile* and disease
piglets (Alvarez-Perez et al., 2009)
calves (Rodriguez-Palacios et al., 2006; Hammitt et al., 2007)

Young animals are often associated with *C. difficile*

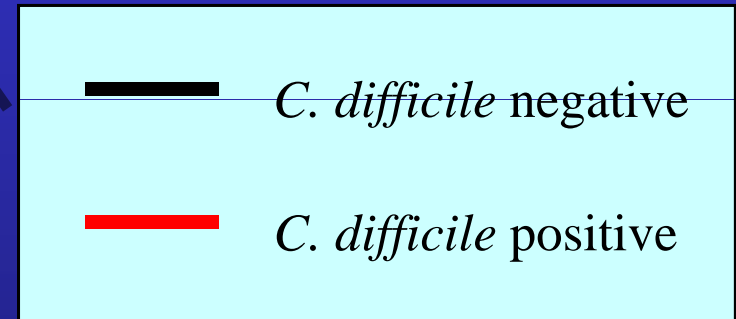
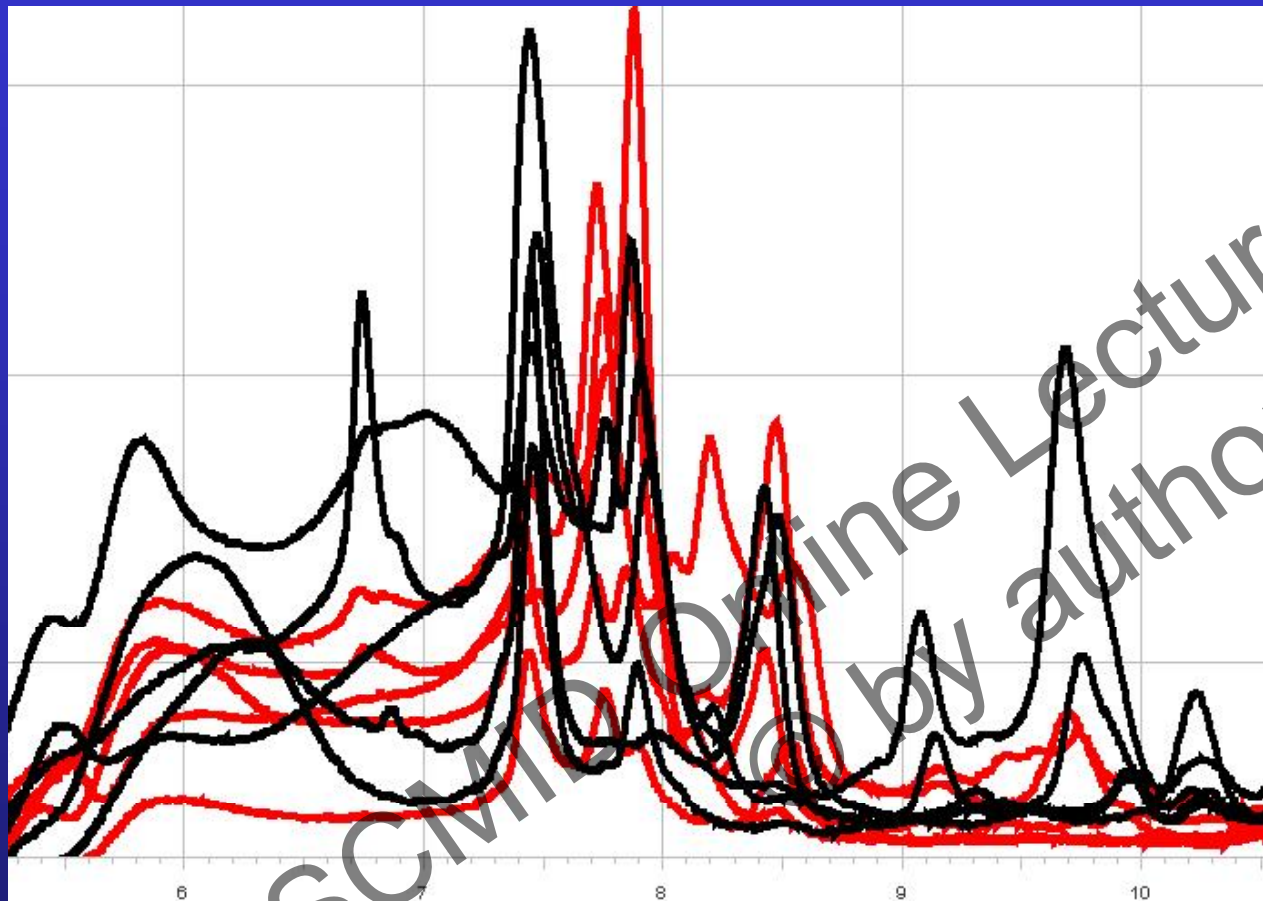
Horse farm - soil samples (Baverud et al., Equine Vet J, 2003)

	% culture positive	num of toxin positive isolates
stud farms	11	10 out of 14
stables with mature horses	1	2 out of 2

Poultry farm – fecal samples (Zidaric et al., Anaerobe, 2008)

animals age	% of positive samples	num. of ribotypes
1 day	0	-
2 weeks	100 %	9
14 weeks	71,4 %	4
18 weeks	40,9 %	6

Analysis of gut microbiota - poultry



DHPLC analysis of total gut eubacterial 16S RNA genes

Lactobacillus

Enterococcus

bifidobacteria

7FP Hyperdiff project (www.clostridia.net/hyperdiff)

J. Skraban, M. Rupnik et al, unpublished

C. difficile - detection in animals

- Culture
enrichment used in most reports
- Commercial toxin tests - low concordance between toxin testing and culture testing

of toxin positive samples

44,8 % were culture +

55,1 % were culture -

toxin positive and culture positive

4,2 % in diarrheic animals

5,2 % in control animals

C. difficile types in humans and animals

host	number of ribotypes found	most prevalent ribotypes or toxinotypes	references
humans	app. 200	0/001; 0/014; III/027; 0/20; VIII/017 (V/078)	Barbut et al., 2007
horses	10 to 12	(V/078 (up to 35%))	Keel et al., 2007; Arroyo et al., 2005
calves	3 to 8	V/078 (up to 94%)	Rodriguez-Palacios et al., 2006; Hammitt et al., 2008 Keel et al., 2007
piglets	2 to 4	V/078 (up to 83%) USA V/066 (up to 67%) Slovenia	Keel et al., 2007 Avbersek et al., 2009

Animals - prevalent types are different in different countries

- Calves

Canada (Rodruigez-Palacios et al., 2006)

017/VIII (29 %), 078/V (25,8 %), 027/III, 014/0, 077/0, 033/XI

USA (Keel et al., 2007)

078/V (94 %), 033/XI, 002/0

- Piglets

USA (Keel et al., 2007)

078/V (83 %), 126/V, 002/0, 033/XI

USA (Baker et al., 2010)

nd/V (99 %),

The Netherlands (Debast et al., 2009)

078/V

Slovenia (Pirs et al., 2008)

066/V, nd/0

Types in humans and animals are indistinguishable

- calves (Canada) (Rodruigez-Palacios et al., Emerg Infect.Dis., 2006)
 - 8 ribotypes
 - 7 of them also in human isolates (same time/geogr. area)
 - 078 (V), 017 (VIII), 027 (III), 033 (XI), 077 (0), 014 (0)
- calves and pigs (USA) (Keel et al., 2007)
 - 4 ribotypes
 - all of them known in human isolates
 - 078 (V), 017 (VIII), 033 (XI), 002 (0), 126 (ND)
- pigs (The Netherlands) (Goorhuis et al., 2008)
 - ribotype 078
 - also emerging highly virulent type in human infections
 - pig and human strains can be identical with MLVA

Contact - *C. difficile* in dogs

C. difficile carriage rate

visitation dogs (healthcare facilities)	28 %
visitation dogs (non-healthcare facilities)	15 %
dogs in households	2,3 %

Type BI/NAP1/027 isolated from healthy visitation dog
(Lefebvre et al., 2006)

C. difficile in meat – where and at what percentage

food	country	% of positive samples	references
ground meat (beef and/or pork)	Canada	20% 6,7 % 12 %	Rodriguez-Palacios et al., 2006 Rodriguez-Palacios et al., 2009 Weese et al., 2010
ground meat (chicken)	Canada	12,8 %	Weese et al., 2010
ground meat (beef and/or pork)	USA	50 %	Songer et al., 2009
ground meat (beef and pork)	Austria	3 %	Jobstl et al., 2010
ground beef	France	2 %	Bouttier et al., 2010
ground beef	Sweden	2,4 %	von Arbercron et al., 2009

C. difficile in food other than meat

- Salads

ready -to-eat salads 7,5% (Bakri et al., 2009)

Scotland, but none of the products originated in the UK

- Milk

raw milk samples, Austria, 0 % (Jobstl et al., 2010)

- Raw vegetables

cucumber, onion, potato, mushroom, carrot, radish (Al Saif and Brazier, 1996)

C. difficile as foodborne infection?

- number of spores
 - from 20 to 240 spores / g of meat (Weese et al., 2009)
 - infection dose not known
- temperature stability of spores at recommended cooking temperature for meat (Rodriguez-Palacios et al., 2007)
- ready to eat products
 - meat products (sausages...) positive in 36,9 % (Songer et al., 2009)
 - salads, raw vegetables

C. difficile genotypes found in food

food	country	% of positive samples	Genotypes
ground meat (beef and/or pork)	Canada	20% 6,7 % 12 %	M26/tox-, 077, 014,M31 M26/tox-, 077, 014,M31,J, C, F 078, 027
ground meat (chicken)	Canada	12,8 %	078
ground meat (beef and/or pork)	USA	50 %	027, 078
ground meat (beef and pork)	Austria	3 %	AI-57, 053
ground beef	France	3 %	(not 078, not 027)
ground beef	Sweden	2,4 %	nd

Summary

- *C. difficile* is a primary pathogen in some animals
- animals can be multiplying host for the microorganism and source for human infection
- transmission from/to animal reservoir can be via contact, environment or food