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Prevalence and typing of *C. difficile* in a group of white veal calves

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Clostridium difficile in food animals

- symptomatic and asymptomatic (cattle, pigs, poultry, horses)
- diversity:
 - animals low (up to 16 PCR ribotypes)
 - human high (app. 200 PCR ribotypes)
- toxinotype V/078 present in high proportion

Host	Number of PCR ribotypes found	Proportion of V/078 isolates	References
calves	3 to 8	up to 94%	Rodriguez-Palacios <i>et al.</i> , 2006; Hammit <i>et al.</i> , 2007; Keel <i>et al.</i> , 2007
piglets	2 to 4	up to 83%	Keel <i>et al.</i> , 2007; Pirs <i>et al.</i> , 2008
horses	10 to 12	up to 35%	Keel <i>et al.</i> , 2007; Arroyo <i>et al.</i> , 2005 Marks and Rupnik, unpublished
poultry	12	no V/078	Zidaric <i>et al.</i> , 2008

Material and methods

Sampling:

- 50 randomly selected individually housed calves monitored for 6 month period (from February to August 2010)
- rectal swabs collected, rectal temperature and fecal score recorded



Isolation:

enrichment broth (CDA supplemented with cycloserine and ceftiofur;
Oxoid)



alcohol shock



selective plate CLO (BioMerieux)



1-4 colonies with morphology of *C.difficile* transferred to fresh COH plate
(BioMerieux)

Identification:

- on basis of colony morphology and typical odour



- molecular confirmation of identification (PCR amplification of species specific *cdd3* gene)

Characterization of the strains:

- toxinotyping and binary toxin CDT
- PCR ribotyping

Results

- at 6 different samplings 300 calf samples collected
- out of 250 collected samples (samplings 1-5) altogether 8,4 % *C. difficile* positive

Sampling point	1 (at arrival)	2	3	4	5	6
Age of calves at sampling point (days)	14	18	25	32	46	194
<i>C. difficile</i> positive samples (%)	10,0%	16,0%	12,0%	2,0%	2,0%	0,0%
Toxinotype/ PCR ribotype	V/078/126, V/066, XI/033, XI/SLO 060 (033-like)	V/078/126, V/066, tox-/SLO 010	V/078/126, 0/012	V/078/126, 0/012	V/078/126	Not applicable

C. difficile positive calves (A-O)

Calf	Sampling point 1 (14 days) Amoxicilline, colistin	Sampling point 2 (18 days)	Sampling point 3 (25 days)	Sampling point 4 (32 days)	Sampling point 5 (46 days) doksiciklin	Sampling point 6 (194 days)
A (5347)			V, BTb+/ 078/126			
B (0346)		tox-/SLO 010				
C (8290)		V, BTb+/ 078/126				
D (0369)	XI, BTb+/SLO 060 (033-like) V, BTb+/066	V, BTb/066				
E (9436)	V, BTb+/ 078/126	V, BTb+/ 078/126				
F (6948)	XI, BTb+/033					
G (7826)	XI, BTb+/SLO 060 (033-like)					
H (53897)			0/012	V, BTb+/ 078/126 0/012		
I (1662)			V, BTb+/ 078/126			
J (63595)		V, BTb/066				
K (3162)		V, BTb+/ 078/126				
L (4167)		V, BTb+/ 078/126				
M (8086)			V, BTb+/ 078/126		V, BTb+/ 078/126	
N (4894)			V, BTb+/ 078/126			
O (3979)	V, BTb+/ 078/126	V, BTb+/ 078/126	V, BTb+/ 078/126			

A role of *C. difficile* in calf enteritis

- *C. difficile* often present in healthy calves

		Diarrheic	Nondiarrheic
This study		6 animals	15 animals
< 7 weeks	culture+	28,6%	78,4%
Rodriguez-Palacios <i>et al.</i> , 2006		144 animals	134 animals
< 4 weeks	culture +	7,6 %	14,9 %
	toxin +	39,6 %	20,9 %
Hammitt <i>et al.</i> , 2007		253 animals	53 animals
< 6 weeks	culture +	25,3 %	13,2 %
	toxin +	22,9 %	30,2 %

Prevalence of *C. difficile* in calves

Age	Prevalence of <i>C. difficile</i>	Country	References
14 days- 46 days	8,4 % (2,0 % - 16,0 %)	Belgium	This study
6 months	0,0 %		
5 days-1 month	11,2 %	Canada	Rodriguez-Palacios <i>et al.</i> , 2006
14 days	33,0 %- 34,0%	Canada	Costa <i>et al.</i> , 2009
16 days	49,0 %- 61,0 %		
1 day- 42 days	13,2 % - 25,3 %	USA	Hammit <i>et al.</i> , 2007
4-6 months	0,5 %	Switzerland	Hoffer, 2010

- **low occurrence** of *C. difficile* in samples taken **before the slaughter** correlates with results obtained by Hoffer 2010 in Switzerland:
 - number of *C. difficile* positive samples (type): 1/204 (078/V)

C. difficile in beef meat and meat products

- **Canada**

1. *C. difficile* isolated from **20,0 %** of ground meat samples; PCR ribotypes **014/0, M31/III, M26/tox-** (Rodriguez-Palacios *et al.*, 2006)
2. *C. difficile* isolated from **12,0 %** of ground beef samples and **4,6 %** veal chop **027, 077, 014** (Rodriguez-Palacios *et al.*, 2009)
3. *C. difficile* isolated from **12,0 %** ground beef; PCR ribotypes **078, 027** (Weese *et al.*, 2010)

- **USA**

C. difficile isolated from **50,0 %** of ground beef; PCR ribotypes **078, 027** (Songer *et al.*, 2009)

- **EU**

1. Austria

C. difficile **not isolated** from beef (Indra *et al.*, 2009)

2. Sweden

C. difficile isolated from **2,4%** ground beef samples; **A+B+ isolates** (von Abercron *et al.*, 2009)

3. France

C. difficile isolated from **3,0%** ground beef, **toxintype 0** (Bouttier *et al.*, 2007)

Prevalent types in calves are changing with geographic region

Belgium

078/126/ V (68,4%),
066/V (10,5%),
012/0 (7,9%),
SLO 010/tox- (5,3%),
SLO 060 (033-like)/XI (5,3%),
033/XI (2,6%)

Canada (Rodruigez-Palacios *et al.*, 2006)

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

USA (Keel *et al.*, 2007)

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

Slovenia (Avbersek *et al.*, 2009)

014/020/0 (50,0 %),
002/0,
033/XIa

Summary

- age dependent colonization with *C. difficile* occurs in both, healthy and diarrheic veal calves
- one genotype (V/078/126) was selected on a single calf farm during certain time period