CLOSTRIDIUM DIFFICILE IN GOATS AND SHEEP IN SLOVENIA

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Introduction

Clostridium difficile appears to be an important cause of enteric disease in different animal species, although high percentage of asymptomatic carriage is reported. To the best of our knowledge, the presence of *C. difficile* in sheep was mentioned only in a single study. The aim of this work was to screen sheep and goats (young and adult animals) for *C. difficile* on eleven different farms in Slovenia.

Materials and methods

Collection of faecal samples or rectal swabs:

Goats

- adult (n=10)
- three to 14 days old (n=32)
- 15 days to one month old (n=21)
- one to four months old (n=66)

Sheep

- adult (n=27)
- one to 16 days old (n=40)
- one to three months old (n=18)

No goat and 24.7 % sheep had diarrhea.

Figure 1 and 2: Goats and sheep sampled in this study

Bacteriological cultivation: cycloserine-cefoxitin fructose enrichment broth supplemented with 0.1% sodium taurocholate
Identification of isolates: morphological criteria and confirmation by multiplex PCR targeting *tpi*, *tcdA*, and *tcdB*
Molecular characterization of isolates: toxinotyping and PCR ribotyping

Results

Table 1: Recovery and strain characterization of *Clostridium difficile* isolates from goats

age groups	adult	3-14 days	15 days-1 month	1-4 months
total number of collected samples	10	32	21	66
number of positive samples	0	6 (18.8 %)	0	1 (1.5 %)*
toxinotype/PCR ribotype		0/SLO 151 (33 %) V/045 (67 %)		tox-**/010 (100 %)* 0/014/020 (100 %)*

* we isolated two different strains from one animal
** tox-: non-toxigenic strain

Table 2: Recovery and strain characterization of *Clostridium difficile* isolates from sheep

age groups	adult	1-16 days	1-3 months
total number of collected samples	27	40	80
number of positive samples	0	2 (5 %)	0
toxinotype/PCR ribotype		XII/056 (50 %) XIa/SLO 061 (50 %)	

All positive animals were asymptomatic.

Conclusions

The results of the present study indicate that domestic small ruminants may also be the reservoir of *C. difficile* and are colonized with similar ribotypes as other animals or humans. PCR ribotype 014/020 was also isolated from poultry, calves, dog and the environment in Slovenia, while PCR ribotype 045 is prevalent on pig farms in central Slovenia. Furthermore, PCR ribotypes 045, 010, 014/020 and 056 were also isolated from humans in Slovenia. We isolated *C. difficile* from very young goats and sheep, which is in accordance with previously published studies of other animal species.

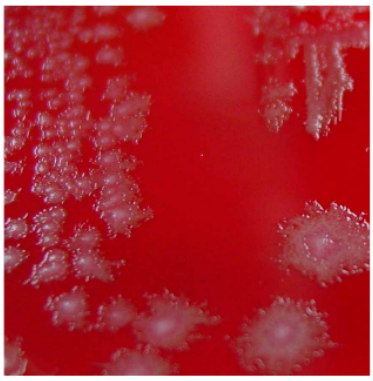

Figure 3: *Clostridium difficile* colonies on cycloserine cefoxitin fructose agar

DETECTION OF *CLOSTRIDIUM DIFFICILE* IN ANIMALS: COMPARISON OF LIGHTCYCLER AND TAQ-MAN REAL-TIME PCR WITH THE CULTURE METHOD

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Introduction

Clostridium difficile-associated disease or asymptomatic carriage has been described for numerous animal species. Variant *C. difficile* strains with binary toxin CDT are often isolated from animals and it seems to be associated with community-acquired *C. difficile* infections in humans. A rapid, simple, sensitive method, capable of detection variant strains, is required for laboratory detection of *C. difficile* in animals. In this study, we compared a TaqMan-based real-time PCR (TM) to a LightCycler real-time PCR (LC), both targeting genes for toxins A, B, and binary toxin. Additionally, both rtPCR assays were compared with the enrichment culture method. Used rtPCR assays are based on different chemistries; hydrolysis vs. hybridization detection probes.

Materials and methods

DNA extraction: from rectal swabs (piglets, n=285) and faecal samples (calves, n=51; foals, n=4) - using QIAamp DNA Stool Mini Kit (Qiagen, Germany)
TaqMan-based real-time PCR: targeting *tcdA*, *tcdB*, *cdtB* genes (Avbersek et al., 2011)
LightCycler real-time PCR: targeting *tcdA*, *tcdB*, *cdtB* genes

Results

Table 1: Comparison of both real-time PCR assays with enrichment culture for detection of toxigenic *C. difficile* in rectal swabs and faecal samples

	real-time PCR positive		real-time PCR negative		real-time PCR unresolved[1]	
	TM	LC	TM	LC	TM	LC
culture positive	73 (21.5 %)	70 (20.6 %)	40 (11.8 %)	39 (11.5 %)	4 (1.2 %)	8 (2.4 %)
culture negative	183 (53.8 %)	185 (54.4 %)	40 (11.8 %)[2]	38 (11.2 %)[3]	0	0

[1] samples were not in complete concordance with A+B+CDT+ type defined for *C. difficile* isolates
[2] inhibition was not observed
[3] PCR inhibition was observed in 2.2 % samples

Concordance between the TM and LC rtPCR was 97.6 %.

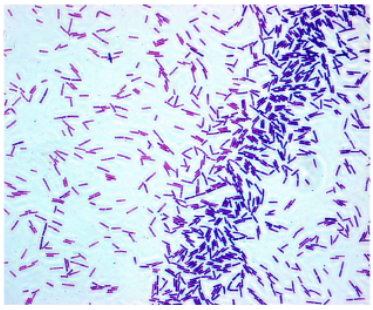

Figure 1: Gram stain of *Clostridium difficile*

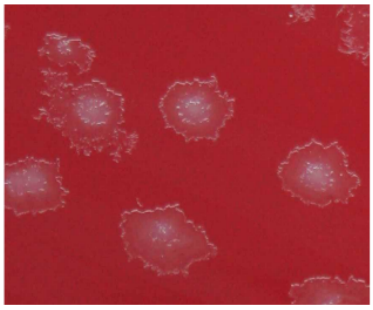

Figure 2: *Clostridium difficile* colonies on cycloserine cefoxitin fructose agar

Conclusions

- Culture-positive/rtPCR negative results could be connected with the low number of *C. difficile* cells in faecal samples or with DNA extraction failure from spores.
- Culture-negative/rtPCR positive results were not regarded as false positive as every sample was tested three times (detection of three genes) with both rtPCR, expect two samples with different results with LC and TM assay. In these cases, contamination could be the reason of such results.
- Theoretically, LC should be more specific assay, while using two specific fluorescent probes instead of one probe used in TM assay but our results showed that both assays gave almost the same results.
- Both assays could be used for screening method but further testing of DNA extraction methods is required to reduce number of samples with false-negative rtPCR result.

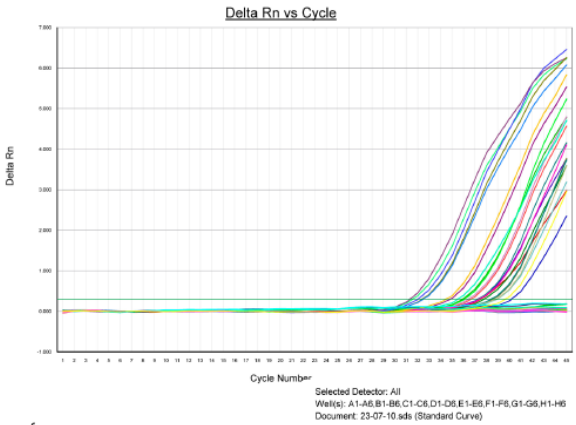

Figure 4: Results of DNA amplification by real-time PCR

References

Avbersek J, Cotman M, Ocepek M. Detection of *Clostridium difficile* in animals: comparison of real-time PCR assays with the culture method. J Med Microbiol 2011; 60: 1119-25.