COMPARISON OF A TAQMAN-BASED REAL-TIME PCR ASSAY TO A COMMERCIAL PCRFAST *CLOSTRIDIUM DIFFICILE* A/B TEST FOR DETECTION OF TOXIGENIC *CLOSTRIDIUM DIFFICILE* IN ANIMALS

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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 new TaqMan-based real-time PCR (TMrtPCR) targeting genes for toxins A, B, and binary toxin to a commercial PCRFast *Clostridium difficile* A/B test (ifp Institut für Produktqualitet, Germany) (PCRFast).

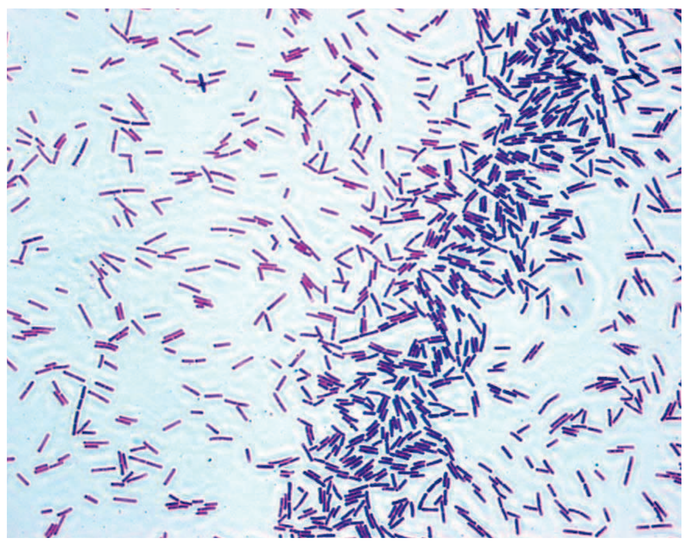

Figure 1: Gram stain of *Clostridium difficile*

Materials and methods

DNA isolated from ten toxinotypes of C. difficile:
toxinotypes 0, I, IIIb, IV, V, VI, VIII, X, XIa, XII

DNA isolation: from rectal swabs (piglets, n=68) and stool samples (calves, n=8) - QIAamp DNA Stool Mini Kit (Qiagen, Germany)

TaqMan-based real-time PCR: targeting *tcdA, tcdB*, and *cdtB* genes; TaqMan Universal PCR Master Mix (Applied Biosystems, USA) - ABI Prism 7000 instrument (Applied Biosystems, USA)

PCRFast Clostridium difficile A/B test: targeting *tcdA* and *tcdB* genes; TaqMan Universal PCR Master Mix - ABI Prism 7000 instrument

Figure 2 and 3: Piglets and calves sampled in this study

Results

Table 1: Results of toxin genes amplification of different *Clostridium difficile* toxinotypes used for validation of real-time PCR

toxinotype	0	I	IIIb	IV	V	VI	VIII	X	XIa	XII
toxin production	A+B+CDT-	A+B+CDT-	A+B+CDT+	A+B+CDT+	A+B+CDT+	A+B+CDT+	A-B+CDT-	A-B+CDT+	A-B-CDT+	A+B+CDT-
TMrtPCR*	A+B+CDT-	A+B+CDT-	A+B+CDT+	A+B+CDT+	A+B+CDT+	A+B+CDT+	A+B+CDT-	A-B+CDT+	A+B-CDT+	A+B+CDT-
PCRFast	pos	pos	pos	pos	pos	pos	pos	pos	neg	pos

* detection of genes coding for toxin A, toxin B, and binary toxin

Table 2: Comparison of real-time PCR results for detection of *Clostridium difficile* in rectal swabs and stool samples

	PCRFast positive	PCRFast negative
TMrtPCR positive*	**8**	**30**
TMrtPCR negative	**0**	**38****

* samples were defined as positive, if test was positive for all genes previously detected by other methods (data not shown)
** in four samples PCR inhibition were observed (internal control negative)

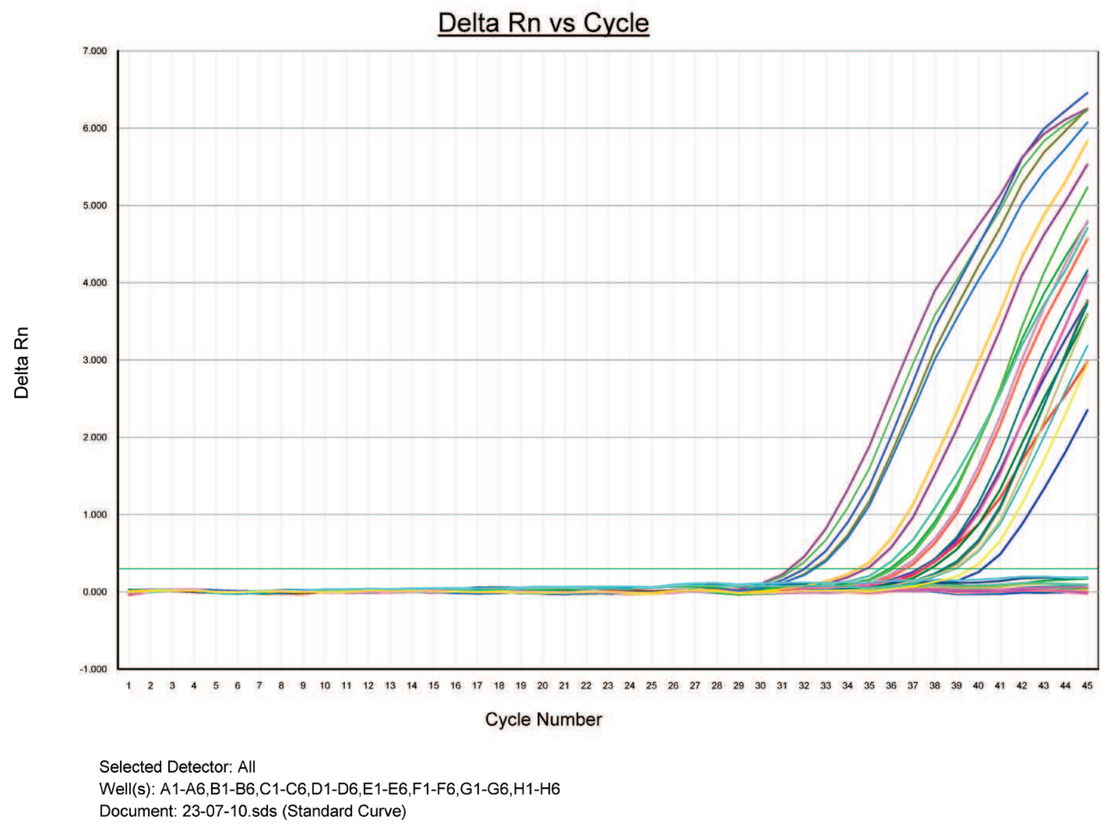

Figure 4: Results of DNA amplification by real-time PCR

Conclusions

In case of toxinotypes VIII and XIa the TMrtPCR assay targeting *tcdA* was positive, while these toxinotypes have gene for toxin A, but it is not expressed. With TMrtPCR we perform triple test for one sample, while in PCRFast test the primers and probes for both genes are mixed in one reaction and it is not possible to distinguish between A-B+ and A+B+ strains. Furthermore, PCRFast test does not detect gene for binary toxin, which is often present in animals. These preliminary results indicate that TMrtPCR is more suitable than PCRFast. Beside this, manufacturer of PCRFast does not specify, that kit is validated on animal samples. In spite of these results further testing of PCRFast with other PCR reagents is required.

COLONIZATION RATES OF *CLOSTRIDIUM DIFFICILE* IN BROILER PRODUCTION CYCLE

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Introduction

Clostridium difficile appears to be an important cause of enteric disease in different animal species. Recently published studies suggest that poultry can be an important reservoir for *C. difficile* with high colonization rates (up to 62%) and high diversity of genotypes. Chicken meat may also be the vehicle for transmission to humans and consequently potential route of infection in case of community-acquired *C. difficile* infections. The aim of this study was to screen for *C. difficile* during a broiler production cycle on one large farm in Slovenia.

Materials and methods

Specimen collection: fecal samples from breeding flock (n=30) and three age groups of broiler chickens originating from the same breeding flock - one day old (n=30), 16 days old (n=30), and 32 days old broilers before slaughter (n=30); broiler neck skins (n=10) from slaughter line

Bacteriological cultivation: cycloserine-cefoxitin fructose enrichment broth supplemented with 0.1% sodium taurocholate (Arroyo et al., 2005)

Identification of isolates: morphological criteria, confirmation by multiplex PCR targeting *tpi, tcdA,* and *tcdB* (Lemee et al., 2004)

Binary toxin gene detection: in-house LightCycler real-time PCR

Figure 1: Broiler chickens sampled in this study

Results

	breeding flock	one day broilers	16 days broilers	32 days broilers	broiler neck skins
number of positive samples	0	0	12 (40%)	2 (6.7%)	0
types of C. difficile isolates			tox- *(6 isolates) A+B+CDT- (7 isolates)**	tox- (1 isolate) A+B+CDT- (1 isolate)	

* tox-: non-toxigenic strain
** from one sample we isolated nontoxigenic and toxigenic strain of C. difficile

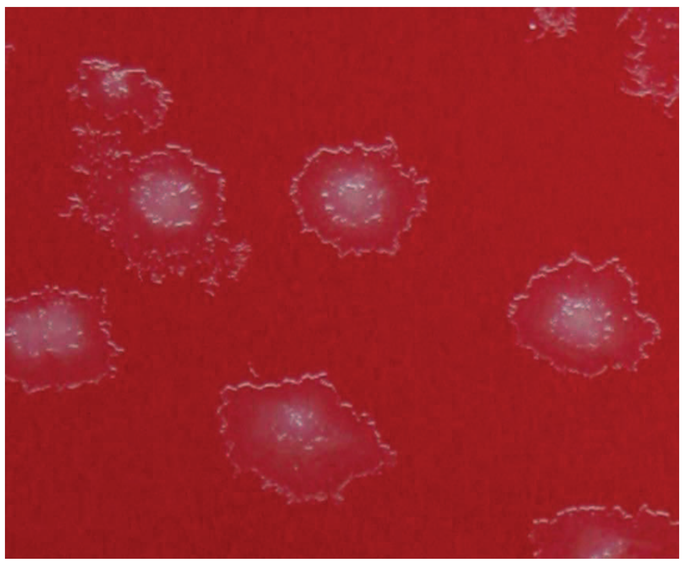

Figure 2: *Clostridium difficile* colonies on cycloserine cefoxitin fructose agar

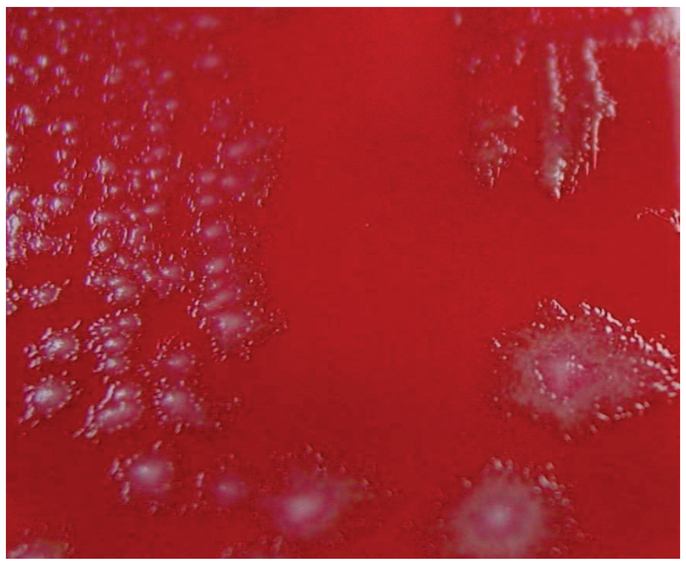

Figure 3: *Clostridium difficile* colonies on blood agar

Figure 4: Microscope image of *Clostridium difficile*

Conclusions

The results of the present study indicate that broiler chickens are colonized in different proportions regarding age. The colonization rate decreased from 40% to 6.7% in two weeks. These could explain previously reported low levels of *C. difficile* in retail chicken meat. We can assume that breeding flock is not the source of *C. difficile* for broiler chickens. Possible source of *C. difficile* could be environment, feed and water. Further studies are needed to get better insight into epidemiology and transmission of *C. difficile* in poultry.

References

Arroyo LG, Rousseau J, Willey BM, et al. Use of a selective enrichment broth to recover *Clostridium difficile* from stool swabs stored under different conditions. J Clin Microbiol 2005; 43:5341-3.

Lemee L, Dhalluin A, Testelin S, et al. Multiplex PCR targeting tpi (triose phospate isomerase), *tcdA* (toxin A), and *tcdB* (toxin B) genes for toxigenic culture of *Clostridium difficile*. J Clin Micobiol 2004; 42:5710-4.