Clostridium difficile in farm and slaughterhouse animals in Belgium: Detection and Characterization

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INTRODUCTION

Clostridium difficile is an anaerobic Gram-positive spore-forming bacteria identified as a severe pathogenic agent in animals and humans. Its recent isolation in foods opens up the occurrence of alimentary origin infections.

OBJECTIVES

Determinate if there is a reservoir of such bacteria in animals engaged to human food and consequent true risk of transmission to humans through the food chain.

Figure 1. Animal samples

MATERIALS AND METHODS

Samples
A total of 437 faecal samples were analyzed. Stools (202 from cattle and 194 from pigs) were collected at the slaughterhouse and from breeding farms (18 from calves and 23 from piglets).

Microbiological analysis
One gramme of each sample was inoculated into 9 ml of cycloserine cefoxitin fructose taurocholate enrichment home made broth and incubated anaerobically for 72h at 37˚C. Subsequently, an aliquot of the broth was spread out on CCFAT and incubated at 37˚C for two days. Additionally, 15 stools from pigs at slaughterhouse were also enriched for a month before plating.

Identification and characterization
All morphological suspected colonies (brown-grey, swarming and typical horse manure-like smell) were verified using a *Clostridium difficile* latex agglutination rapid test (Oxoid) and subcultures onto blood agar (BioRad) (Figure 2&3). Isolates were identified by PCR detection of *tpi*, *tcdA*, *tcdB* and *cdtA* genes. Toxic activity was confirmed by a fecal cytotoxin inmmunoessay. Further characterization was performed by PCR ribotyping.

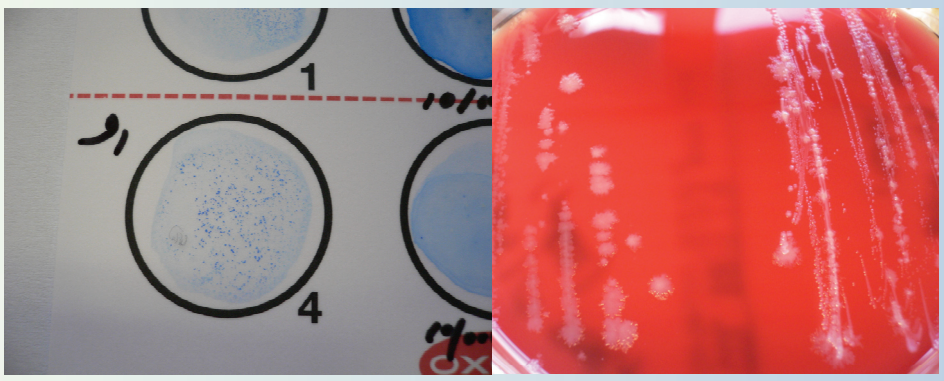

Figure 2. Latex agglutination assay and subculture onto blood agar

RESULTS

Clostridium difficile was isolated from 8.9% (39/437) of the total samples: 14 from cattle at slaughterhouse (6.9%), 18 from piglets (78.3%) at farm and 4 from calves (22.2%) at breeding farms. None of the pig fecal samples at slaughterhouse was positive for *Clostridium difficile* after three days of enrichment but 3/15 tested positive after one month of enrichment. Thirty-four of the total strains were toxin positive (Table 1). Eighteen different PCR ribotypes were identified with type 078 predominant in breeding farms of calves (75%) and piglets (66.7%). Isolates from cattle presented the widest range in ribotypical variety.

Animal Group	PCR-Ribotype	n	Cytotoxin -assay	*tcdA*	*tcdB*	*cdtA*
Piglets	078	12	+	+	+	+
	002	3	+	-	+	+
	172 UCL	1	+	+	+	-
	239 UCL	1	+	-	+	+
	9 UCL	1	-	-	-	-
Calves	078	3	+	+	+	+
	015	1	+	+	+	-
Cattle	002	1	+	-	+	-
	014	1	+	+	+	-
	081	1	+	+	+	-
	087	1	+	+	+	-
	103 UCL	1	-	-	-	-
	118 UCL	1	+	+	+	-
	36 UCL	1	-	-	-	-
	16 UCL	1	+	+	+	-
	16r UCL	1	+	+	+	-
	118a UCL	2	+	+	+	-
	20a UCL	1	+	+	+	-
	238 UCL	1	-	-	-	-
	273 UCL	1	-	-	-	-
Pigs	078	2	+	+	+	+
	081	1	+	-	-	-

Table 1. PCR-ribotypes and toxin gene profiles of Clostridium difficile isolated from farm and slaughter animals

CONCLUSIONS

Present study proves the presence of *Clostridium difficile* in animals engaged to food in Belgium. The presence of the bacteria at the slaughterhouse and the high prevalence of pathogenic strains states a true risk of contamination to humans through the food chain.

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Figure 3. Identification of *Clostridium difficile* by morphological criteria