Clostridium difficile in poultry production: detection and characterisation

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Introduction

- The finding of *Clostridium difficile* in major food animals and retail meat suggests a possible animal reservoir for community acquired *Clostridium difficile* infection (CDI). The objective of this study was to evaluate the presence and characteristics of *Clostridium difficile* in poultry production in Slovenia.

Material and methods

- A total of 880 samples from broiler farms (n=610) and laying hen farm (n=270) were collected from 7 different locations :
  - broiler farms: faecal samples (n=540), slaughter line samples (n=46); environmental samples (n=24)
  - laying hen farm: faecal samples (n=255); environmental samples (n=15)
  - Faecal samples were collected subsequently from breeding flocks and then at different age of the progeny.
- Bacteriological cultivation: cyloserine-cefoxitin fructose enrichment broth supplemented with 0.1% sodium taurocholate, alcohol shock treatment, solid selective medium (Arroyo et al., 2005)
- Identification: colonies were chosen based on morphological criteria and typical odour, confirmed by multiplex PCR targeting *tpi*, *tcdA*, and *tcdB* (Lemee et al., 2004)
- Characterisation: PCR ribotyping (Bidet et al., 1999) and toxinotyping (http://www.mf.uni-mb.si/tox). One and up to three strains per animal were ribotyped; toxinotyping was performed on randomly selected representatives of each ribotype.

Figure 1: Slovenia - the location of poultry farms included in the study.

Results

Clostridium difficile was isolated from 189/880 (21.5 %) samples from 6/7 (85.7 %) farms, see Table 1.

Table 1 The number and types of *C. difficile* strains isolated from poultry

	farm	culture positive (%)							PCR ribotypes/toxinotypes*
		breeding flock	1-3 days	6 - 8 days	11 – 18 days	21- 25 days	before slaughtering	slaughter line / before laying	
broilers	A	3/25 (12 %) (common for farms A, B, C)	1/10 (10 %)	0/15 (0 %)	0/20 (0 %)	0/10 (0 %)	0/20 (0 %)	0/12 (0 %)	**014/020/0, 045/V, 002/0**
	B		1/10 (10 %)	2/15 (13.3 %)	0/20 (0 %)	1/10 (10 %)	1/20 (5 %)	0/12 (0 %)	**014/020/0, 002/0,** SLO 160/0
	C		1/10 (10 %)	2/15 (13.3 %)	1/20 (5 %)	0/10 (0 %)	0/20 (0 %)	1/12 (8.3 %)	**014/020/0, 002/0**
	D	0/30 (0 %)	0/30 (0 %)	n	12/30 (40 %)	-	2/30 (6.7 %)	0/10 (0 %)	**001/072/0, 002/0, SLO 080/tox-, SLO 020/0**
	E	1/30 (3.3 %)	-	17/20 (85 %)	14/20 (70 %)	-	1/20 (5 %)	-	**014/020/0, 018/0, 002/0, 046/0, SLO 084/tox-, SLO 034/0, 005/0, 015/0, 011/049/0**
	F	0/30 (0 %)	0/10 (0 %)	0/20 (0 %)	0/20 (0 %)	-	n.a.	-	n.a.
laying hens	G	0/30 (0 %)	0/20 (0 %)	21/30 (70 %)	20/27 (74 %)	-	n.a.	0/30 (0 %) (15 weeks)	**014/020/0, 002/0, 001/072/0, SLO 131/tox-, 046/0, SLO 080/tox-, 056/XII, SLO 084/tox-, 070/0, 010/tox-, 015/0, SLO 064/tox-, SLO 074/0, 005/0, 011/049/0, SLO116/XXIV, 056/XII,** SLO 091/tox-
	G (2nd sampling)	-	-	14/20 (70 %)	15/20 (75 %)	12/20 (60 %) (3 weeks) 17/23 (73.9 %) (4 weeks) 18/20 (90 %) (5 weeks)…			-

* Ribotypes marked in bold are shared with human PCR ribotypes identified in Slovenia (Janežič et al., 2012)

5/24 (20.8%) of environmental samples were positive in broiler farms. On farm F, where all animals were negative, 3/3 (100%) soil samples were positive. On farms A-C, 2/21(9.5 %) samples were positive, one from litter (1/6, 16.6 %) and one from drinking water (1/15, 6.6 %).

In laying hen farm G, 6/15 (40 %) environmental samples were positive. The samples were litter (n=2), drinking water (n=1), soil (n=5), feed (n=1) and disinfectant footbath at the entry (n=1). 2 soil samples and 1 litter sample were positive. This farm has a peculiar feature, there are cows and donkeys grazing on the holding. Their stools were taken as environmental samples (n=6): cow samples were all negative (3/3) and donkey samples were all positive (3/3, 100%).

During processing the flock on slaughter line, the following samples were taken: neck skins, intestine contents, meat samples of carcasses, scalding water, gloves. Only scalding water was positive.

22 different PCR ribotypes were identified that were non-toxigenic or belonged to toxinotypes 0, V, XII and XXIV. The diversity of isolates was high within the farm. Various ribotypes were also found in individual animals. Ribotype 014/020 was the most prevalent strain (30/123; 24.4 %), followed by 002, 001/072 and 046. The most prevalent toxinotype was 0.

Conclusion

Clostridium difficile is common in poultry farms, but the number of isolates at the time of entry into the food chain is low. Interestingly, at the age when the broilers are slaughtered (app. 4-5 weeks), the laying hens were found highly positive (73.9 and 90 %, respectively).
Our results show not only colonisation in different proportions regarding age, but also difference between farms: at age groups where colonisation rates were expected to peak, there was a wide range on different farms (0–85%).

Environmental samples were found positive in the farm with high colonisation rate and this may indicate environmental source of *C. difficile*. However, soil samples were positive in negative farm as well.
The diversity of genotypes was high, which is consistent with the previous reports (Zidarič et al., 2008). The most common PCR ribotypes found in the study, also prevail in humans and environment in our geographic region (Janežič et al., 2012) and this may suggest occurrence of transmission among various reservoirs.

References

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