Prevalence, concentration and molecular epidemiology of *Clostridium difficile* on neonatal calf carcasses in Australia

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BACKGROUND AND AIMS

Clostridium difficile is a ubiquitous, spore forming, Gram positive anaerobe, the leading cause of antimicrobial and health care-associated diarrhoea in humans [1].

Recent reports in Europe and North America of *C. difficile* being isolated from production animals and retail meats have raised concerns about potential risks to public health [2].

Concomitantly, the incidence of *C. difficile* infection in humans has increased in the community, with mounting evidence of a genetic overlap between strains isolated from human and animal sources [2-4].

In a previous study, we found 72% of faecal samples from <7-day old Australian dairy calves at slaughter were positive for *C. difficile* [5]. This current study aims to provide further data on the prevalence, concentration and genotype of *C. difficile* in the faeces and the on carcasses of Australian veal calves at slaughter.

METHODOLOGY

Sampling took place in April 2013 in three abattoirs supplied by farms in SA (n=1) and VIC (n=2). Calves were aged <14 days at slaughter and carcasses were sampled post processing and washing, immediately as they entered the chiller.

Sampling of veal calve carcasses (n=300) occurred in six batches (A-F) and followed the meat industry standard carcass swabbing technique for calves [6] using pre-hydrated and sterilised Polywipes™ (MWE, Corsham, UK) sampling a total carcass area of 75 cm^2. Sampling of veal calve faeces (n=30) was performed as previously described [5].

All samples were transported under ambient conditions to The University of Western Australia, stored at 4ºC and processed within 24 h.

Selective culture (both direct and enrichment) was performed as previously described [7] and viable counts were performed on a proportion of fecal (n=15) and carcass samples (n=150). *C. difficile* isolates were identified and characterised by PCR for toxin genes (tcdA, tcdB and cdtA/B) and PCR ribotyping as previously described [5].

RESULTS AND DISCUSSION

Prevalence

C. difficile was found in animals from all abattoirs sampled, although prevalence varied between sites (Table 1). Overall *C. difficile* prevalence was 25.3% (76/300) on carcasses and 60.0% (18/30) in faeces (Table 1), the latter being consistent with the findings of our earlier study [5]. The majority of isolates (67/82) were recovered by direct culture as well as enrichment, which is indicative of relatively high levels of spores.

The prevalence of *C. difficile* recorded was higher than reported in similar studies overseas: Canada, 11.2% (31/278) [8], the United States 9% (18/50) [9], Slovenia 9% (4/42) [10] and Switzerland 0.5% (1/204) [11]. These differences could be attributable to a number of factors such as seasonality and differences in culture/testing methodology.

Table 1. Recovery of *C. difficile* from carcasses and faeces of veal calves at slaughter

Sample Type	Abattoir	State	Batch ID	N samples	N positive‡ n (%)	Direct and enrichment	Enrichment only	N Isolates‡
Carcasses	I	SA	A	50	29 (58.0)	29	0	34
	II	VIC	B,C	100	32 (32.0)	23	9	33
	III	VIC	D,E	100	10 (10.0)	10	0	10
	I	SA	F	50	5 (10.0)	5	0	5
	Total			**300**	**76 (25.3)**	**67**	**9**	**82**
Faeces	I	SA	A	5	4 (80.0)	0	4	4
	II	VIC	B,C	10	3 (30.0)	1	2	5
	III	VIC	D,E	10	9 (90.0)	6	3	9
	I	SA	F	5	2 (40.0)	1	1	2
	Total			**30**	**18 (60.0)**	**8**	**10**	**20**

‡ some samples contained multiple strains

Viable counts

Of those faecal samples with *C. difficile* levels above the limit of detection (n=10/30), the mean concentration of *C. difficile* in faeces was $2.8x10^5$ cfu/mL (Fig. 1). Of those carcass samples with *C. difficile* levels above the limit of detection (n=25/150), the median count was 3 cfu/cm^2, (highest concentration of 33 cfu/cm^2) (Fig. 2).

To our knowledge this is the first time that *C. difficile* has been isolated from carcasses of Australian veal calves and further confirms our previous finding that the faeces of neonatal veal calves at slaughter contain *C. difficile* [5].

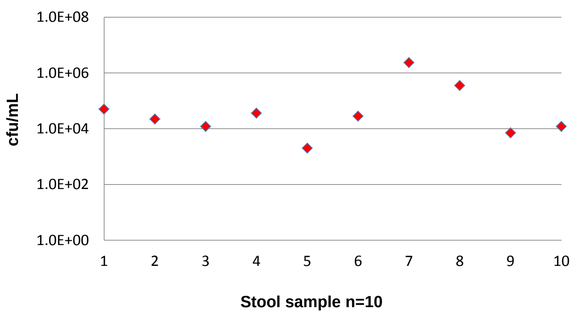


Figure 1. Concentration of viable *C. difficile* in 10 different fecal samples

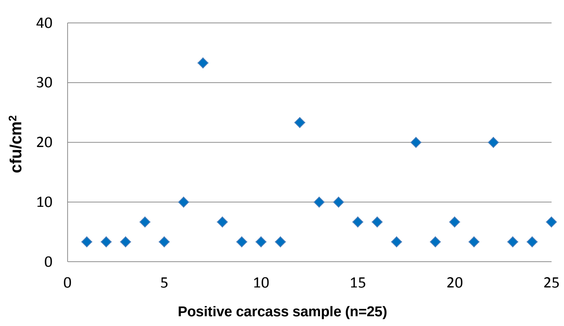
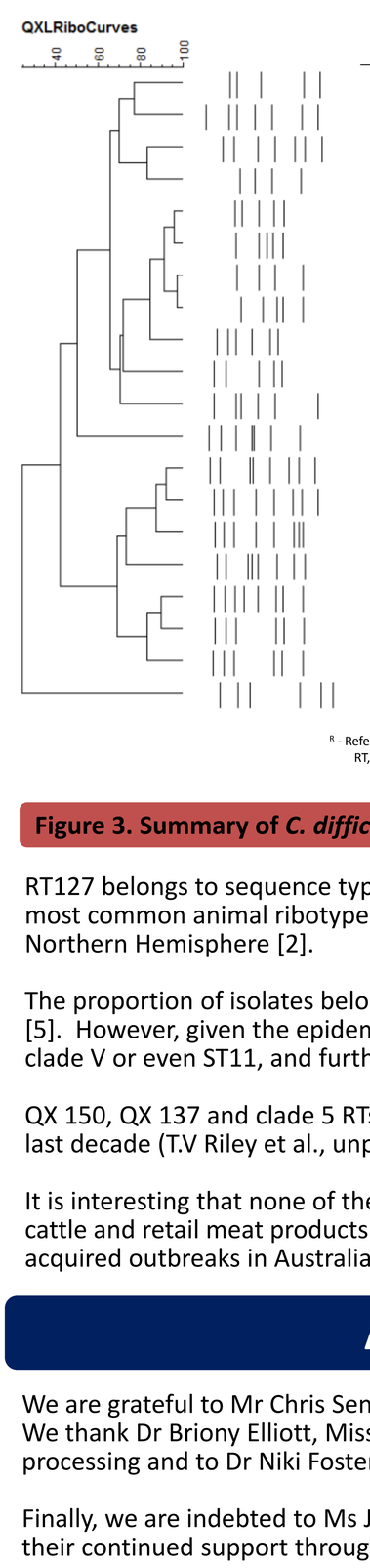


Figure 2. Concentration of viable *C. difficile* in 25 positive carcass samples

Molecular epidemiology

Of the 102 isolates of *C. difficile* recovered from calves, 90 (88.2%) were positive for *tcdA* and *tcdB* (A^+B^+), of which 37 (41%) were also positive for binary toxin genes *cdtA/B* (CDT^+) (Fig. 3).

Multiple PCR ribotypes (RTs) were identified (Fig. 3): 62.7% (n=64) were assigned one of 10 internationally recognised RTs (Fig.3). The most prominent RT was 127 comprising 33.3% of isolates. Ribotypes QX 150 and UK 137 comprised 28.4% and 14.7% of isolates, respectively, and QX 150 was found almost exclusively in the first batch of abattoir I.



PCR Ribotype	Toxin Profile			N (%)
	tcdA	*tcdB*	*cdtA/B*	
QX 141	-	-	-	1 (1.0)
UK 027[R]	+	+	+	
UK 103	+	+	-	1 (1.0)
QX 150	+	+	-	29 (28.4)
UK 126	+	+	+	2 (2.0)
UK 127	+	+	+	34 (33.3)
UK 033	-	-	+	6 (5.9)
QX 233	-	-	-	2 (2.0)
UK 078[R]	+	+	+	
UK 070	+	+	-	1 (1.0)
UK 251	+	+	+	1 (1.0)
QX 231	+	+	-	1 (1.0)
UK 056	+	+	-	2 (2.0)
QX 006	+	+	-	2 (2.0)
UK 052	+	+	-	1 (1.0)
QX 104	-	-	-	1 (1.0)
UK 053	+	+	-	1 (1.0)
QX 234	-	-	-	1 (1.0)
UK 137	+	+	-	15 (14.7)
QX 232	-	-	-	1 (1.0)

[R] - Reference strain, *tcdA* - toxin A, *tcdB* - toxin B, *cdtA/B* - binary toxin (CDT), UK - internationally recognised RT, QX - internal nomenclature. Dendrogram created using BioNumerics as previously described [5]

Figure 3. Summary of *C. difficile* PCR ribotypes and toxin profiles

RT127 belongs to sequence type (ST) 11 (by MLST) which falls into clade 5, the same as RT078, the most common animal ribotype worldwide [3] and increasingly associated with community CDI in the Northern Hemisphere [2].

The proportion of isolates belonging to clade 5 RTs 126 and 033 was lower than in our previous study [5]. However, given the epidemiological link with animals, it is possible that QX 150 also groups within clade V or even ST11, and further research is underway to determine its phylogeny.

QX 150, QX 137 and clade 5 RTs 126, 127 and 033 have been isolated from humans in Australia in the last decade (T.V Riley et al., unpublished data).

It is interesting that none of the ribotypes detected was the same as ribotypes commonly found in cattle and retail meat products overseas, and that strains associated with *C. difficile* community-acquired outbreaks in Australia (RT244, RT251) and overseas (RT 027, RT 078) were not recovered.

CONCLUSIONS

- **The prevalence and viable count data presented here, confirm earlier findings that Australian neonatal calves are a potential source of *C. difficile*.**
- **The faeces and carcasses of a large proportion of the Australian calves sampled in this study are colonised with strains of *C. difficile* known to cause disease in humans, albeit with relatively low numbers of cases currently in Australia.**
- **Typing of *C. difficile* isolates revealed a heterogeneous strain population that contrasts with studies in the Northern Hemisphere where a single well-described RT (078) predominates.**
- **Although not quantified, there remains a risk to public health if *C. difficile* spores on contaminated veal products survive downstream processing and cooking.**

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REFERENCES

1. Rupnik M, Wilcox MH, Gerding DN. 2009. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. Nat. Rev, Microbiol. 7:526-36.
2. Hensgens MP, Keessen EC, Squire MM, Riley TV, Koene MG, Boer Ed, et al. *Clostridium difficile* infection in the community: a zoonotic disease? 2012. Clin. Microbiol. Infect. 18:635-45.
3. Rupnik M, Widmer A, Zimmermann O, Eckert C, Barbut F. 2008. *Clostridium difficile* toxinotype V, ribotype 078, in animals and humans. J. Clin. Microbiol. 46:1963-4.
4. Rupnik M. 2010. *Clostridium difficile*: (Re)emergence of zoonotic Potential. Clin. Infect. Dis. 51:584.
5. Knight DR, Thean S, Putsathit P, Fenwick S, Riley TV. 2013. Cross-sectional study reveals high prevalence of *Clostridium difficile* non-PCR ribotype 078 strains in Australian veal calves at slaughter. Appl. Environ. Microbiol. 79:2630-2635.
6. Australian Quarantine and Inspection Service (AQIS). 2009. Carcase Microbiological Monitoring Program (ESAM). Notice Number MEAT 2000/092000.
7. Knight D, Squire M, Riley T. 2013. Evaluation of diagnostic tests to detect *Clostridium difficile* in piglets. *In* Proceedings of the 8th Int. Conference on the Molecular Biology & Pathogenesis of the Clostridia (CLOSTPATH), Palm Cove QLD, Australia.
8. Rodriguez-Palacios A, Stämpfli HR, Duffield T, Peregrine AS, Trotz-Williams LA, Arroyo LG, et al. 2006. *Clostridium difficile* PCR ribotypes in calves, Canada. Emerg. Infect. Dis. 12:1730-6.
9. Houser BA, Soehnlen MK, Wolfgang DR, Lysczek HR, Burns CM, Jayarao BM. 2012. Prevalence of *Clostridium difficile* toxin genes in the feces of veal calves and incidence of ground veal contamination. Foodborne. Pathog. Dis. 9:32-6.
10. Avbersek J, Janezic S, Pate M, Rupnik M, Zidaric V, Logar K, et al. 2009. Diversity of *Clostridium difficile* in pigs and other animals in Slovenia. Anaerobe. 15:252-5.
11. Hoffer E, Haechler H, Frei R, Stephan R. 2010. Low occurrence of *Clostridium difficile* in fecal samples of healthy calves and pigs at slaughter and in minced meat in Switzerland. J. Food. Prot. 73:973-5.