THE PREVALENCE AND IMPACT OF TETRACYCLINE RESISTANCE IN *CLOSTRIDIUM DIFFICILE* IN CALVES

M Costa*[1], HR Staempfli[1], LG Arroyo[1], DL Pearl[1], JS Weese[1]

[1]University of Guelph, Guelph, Ontario, Canada.

Introduction

- While the role of *C. difficile* as a bovine pathogen is unclear, there is evidence implicating it as a cause of neonatal diarrhea in cattle. Further, the emergence of community-associated *C. difficile* infection and the presence of indistinguishable strains in humans, food animals and food has raised concerns about the potential for zoonotic or foodborne transmission.
- Longitudinal study in some species has demonstrated marked variations in prevalence between different age groups, but this has not been studied in cattle.
- The prophylactic and therapeutic use of antimicrobials is a common practice in food animal production systems, with tetracycline been one of the most used. The impact of routine antimicrobial use on the prevalence of *C. difficile* in calves is not known.

Objectives

- To longitudinally investigate *C. difficile* colonization in veal calves and to characterize recovered isolates.
- To determine the impact of tetracycline treatment on tetracycline resistance and to determine the impact of tetracycline resistance on prevalence of specific strains.

Methods

- One veal farm in Southern Ontario, raising male Holstein calves obtained from multiple dairy farms, was used.
- Calves were 2 to 10 days of age at arrival and were either housed in groups (rooms 1 and 2) or individually (rooms 3 and 4).
- As per standard farm protocols, calves were fed milk replacer with oxytetracycline (22.2 mg/kg/day) for 5 days after arrival to control respiratory diseases.
- Fecal samples were collected within 48h after arrival and repeated after one, 17 and 21 weeks (5 days prior to slaughter).
- Enrichment in *C. difficile* moxalactam-norfloxacin (CDMN) broth with 0.1% taurocholate was followed by inoculation onto CDMN agar after alcohol shock.
- Isolates were characterized by ribotyping (Bidet et al., 1999). All strains, but ribotype 078, were classified with an internal nomenclature.
- PCR detection of *tcd*A, *tcd*B (Lemee et al., 2004), *cdt*A. (Stubbs et al., 2000), *tet*M (Ng et al., 2001) and *tcd*C sequence analysis were performed.
- Tetracycline susceptibility was evaluated using the Etest method (breakpoints: R> ≥16 µg/ml, S<4 µg/ml, I 4-16 µg/ml).
- Multivariable logistic regression models were built to determine the impact of date of sampling, and room on tetracycline resistance and the presence of *tet*(M) gene.
- The correlation between presence of *tet*(M) and phenotypic resistance to tetracycline was calculated by the Spearman's test.

Results

- *Clostridium difficile* was common in calves at arrival and 1 week later (Table 1, Figure 1). There was a significant increase between the 1st 2 samples (OR 1.2, *P*=0.001) and a significant decrease to the 3rd and 4th samples (OR 0.13 and 0.04, respectively, *P*<0.001 for both).
- Ribotype 078 was most common at all time points (Table 2), however there were differences in ribotype distribution over time. Calves were 18 times as likely to shed for ribotype E during 2nd sampling compared to the 1st.

	<48 hours	1 week	17 weeks	21 weeks
Prevalence	56/174 (32%)	88/172 (51%)	4/183 (2%)	4/156 (2%)
Resistance	40/53 (76%)	81/87 (96%)	-	-
tet(M) gene	7/53 (13%)	37/85 (43%)	-	-

Table 1: Overall prevalence of *C. difficile* and prevalence of resistant isolates and *tet(*M) gene carriage

Ribotype	Toxin otype	*tcdA*	*tcdB*	*cdtA*	*tcdC*	<48 h	1 wk	17 wk	21 wk	Overall
078	V	+	+	+	39bp	66%	66%	75%	100%	67%
E	0	+	+	-	-	3.6%	33%	0%	0%	20%
H75	0	+	+	-	-	7.1%	0%	0%	0%	3%
Q	XII	+	+	-	-	5.3%	1%	0%	0%	2%
L	0	+	+	-	-	3.6%	0%	0%	0%	1%
MOH AA	XIII	-	+	-	18bp	1.8%	0%	25%	0%	1%
OVC G	0	+	+	-	-	3.6%	0%	0%	0%	1%
OVC AA	0	+	+	-	18bp	1.8%	0%	0%	0%	0.6%
OVC J	-	-	-	-		1.8%	0%	0%	0%	0.6%
F	0	+	+	-	18bp	1.8%	0%	0%	0%	0.6%
R	0	+	+	-	18bp	1.8%	0%	0%	0%	0.6%
CM 53	-	-	-	-		1.8%	0%	0%	0%	0.6%

Table 2: Molecular characterization of *C. difficile* from veal calves and distribution of the strains over time.

- Tetracycline resistance was common particularly after tetracycline treatment (Figure 2, Table 3)). The MIC_{50} and MIC_{90} were 16 µg/mL and 32 µg/mL, respectively (range 0.25 µg/mL to 48 µg/mL).
- Isolates from the second sampling were almost three times as likely to be resistant and almost five times as likely to possess the *tet*(M) gene when compared to arrival (Table 3).
- *tet*(M) was more common in isolates from calves housed in rooms 3 and 4 compared to room 1.
- Correlation between resistance and the presence of *tet*(M) was weak (*r*=0.16; *P*=0.058), because of isolates that were *tet(*M) negative but resistant.
- 6 susceptible isolates had *tet*(M), but resistance was induced in all by tetracycline exposure.
- Ribotype E was almost 10 times as likely to be resistant when compared to ribotype 078 and 100 times as likely than other strains (Table 3). There was no association between the different ribotypes and the presence of the *tet*(M) gene.

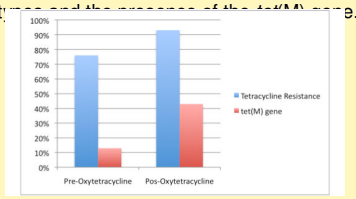

Figure 2: Prevalence of tetracycline resistance before and after after administration of oxytetracycline.

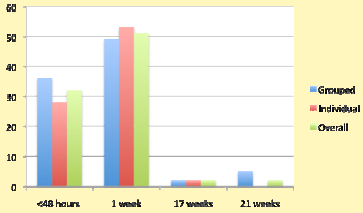

Figure 1: Prevalence (%) of *C. difficile* in veal calves by group.

Discussion

- The high prevalence at arrival was not surprising, as a high prevalence of colonization has been reported in young calves.
- The use of oral oxytetracycline may have contributed to the increase the shed of *C. difficile* during the 2nd sampling.
- The low prevalence before slaughtering may be of importance when evaluating foodborne risks. A decrease in prevalence with increasing age appears to be present with multiple animal species.
- Almost 99% of the toxigenic isolates found here have been identified in humans in the province.
- The dominance of ribotype 078 is a valuable epidemiologic information, as the strain has increasingly been found from clinical cases in humans.
- The increase on the number of isolates carrying the *tet*(M) gene during 2nd sampling. It is reasonable to hypothesize that routine tetracycline use resulted in this effect. The ribotype shift noted by the 2nd sample might be related to antimicrobial selection.
- The low correlation found between resistance and presence of the *tet*(M) gene is suggestive that one or more other factors are involved on resistance. Further study is ongoing.
- The reason why isolates from rooms 3 and 4 were more likely to carry the *tet*(M) gene may be related with the epidemiology of specific strains in those rooms or with external factors.
- Further studies are necessary to address the impact of antimicrobial use on *C. difficile* shedding in food animals.

	Tetracycline resistance			Presence of *tet*(M)		
	OR	*P*>|z|	[95% CI]	OR	*P*>|z|	[95% CI]
Sampling 1	Reference			Reference		
Sampling 2	2.96	0.016	1.2 - 7.16	4.96	0.004	1.65 - 14.91
Room 1	Reference			Reference		
Room 2	0.30	0.059	0.08 - 1.05	3.77	0.085	0.83 - 17.04
Room 3	0.59	0.479	0.14 - 2.51	23.38	0.000	5.11 - 107.02
Room 4	0.45	0.271	0.11 - 1.84	10.87	0.001	2.59 - 45.51
Ribotype E	Reference			Reference		
Ribotype 078	0.09	0.035	0.01 - 0.85	1.93	0.254	0.62 - 6.02
Other	0.01	0.000	0.00 – 0.10	-	-	-

Table 3: Multivariable logistic regression model testing the effect of the sampling time and room of housing on presence of tetracycline resistance and the *tet*(M) gene.

References available upon request.