Introduction

- Clostridium difficile colonization has been reported to be common in calves, yet there has been limited study of older cattle.
- The role of C. difficile in bovine disease is unclear, and the main concerns regarding C. difficile in older cattle are the potential for zoonotic or foodborne infection. This has been heightened by apparent increases in community-associated C. difficile infection in humans and the over-representation of toxigenic V strains that may be common in some food animals.
- C. difficile spores have been recovered from food products including retail meat; however, the source of contamination has not been identified.
- Study of cattle close to the age of slaughter is required for a better understanding of food contamination risks.

Objectives

- To evaluate the shedding of C. difficile by beef cattle upon arrival and exit from feedlots.
- To characterize the isolates that were recovered by PCR ribotyping and pulsed-field gel electrophoresis (PFGE).

Methods

- Four commercial feedlots in Alberta, Canada were enrolled.
- Fecal samples were collected from cattle at the time of arrival to the feedlot (~12 months of age) and then 2 to 3 months later, just before leaving for slaughter.
- Enrichment in C. difficile moxalactam-noroxacin (CDMN) broth with 0.1% taurocholate was followed by alcohol shock and inoculation onto CDMN agar.
- Isolates were characterized by ribotyping (Bidet et al., 1999). If the ribotype pattern was a recognized international ribotype, as determined thorough comparison with a reference strain, it was given the appropriate numerical designation (i.e. 078) Otherwise, an internal nomenclature was used.
- PCR detection of tcdA, tcdB (Lemee et al., 2004) and cdtA (Stubbs et al., 2000) was performed. Another PCR for detection of toxin A gene constitutive difference between A+/B- strains was performed according to Kato et al. (1998).
- The isolates recovered were further characterized by PFGE and tcdC sequence analysis.
- Descriptive statistical analysis was performed and the Fisher’s exact test was used for overall and feedlot prevalence comparison.

Results

- A total of 543 cattle were sampled, ranging from 121 to 179 cattle per feedlot (mean 137).
- Clostridium difficile was isolated from 3.3% (18/543) of cattle at the time of feedlot arrival and from 5.5% (18/330) of cattle before leaving for slaughter (Table 1).
- Overall, there was no difference in the prevalence of C. difficile shedding on arrival versus exit (P=0.161), nor was there any difference at the individual lot level in three of the feedlots (P=0.622; P=1.000; and P=0.385).
- In one feedlot (Feedlot 3), the prevalence was significantly higher at exit (P=0.017), increasing from 2.5% to 11.8%.
- 330 paired samples were available. Most of the animals that were lost to follow up were from feedlot 2, where only 12/121 animals were sampled at exit.
- When only the 330 paired samples were considered, there was a significantly higher overall prevalence at exit compared to arrival (P=0.039).
- Feedlot 3 had a significantly higher prevalence of animals shedding C. difficile at exit compared to arrival (P=0.011), while there was no difference in feedlots 1 and 4 (P=0.370 and P=0.620).
- No individual animal was positive on both arrival and exit.
- All the isolates recovered were classified as ribotype 078 and had tcdA, tcdB and cdtA. A 39bp deletion in tcdC and an upstream C184T nonsense truncating mutation were present.
- All isolates were indistinguishable by PFGE and classified as North American pulsortype 7 (NAP 7) (Figure 1).

Discussion

- The low prevalence of C. difficile in beef cattle reported here is in agreement with another study that reported a prevalence of 4.5% in cows (Indra et al., 2009).
- While C. difficile can be common in healthy calves, these results are consistent with data from longitudinal studies in pigs, whereby there is an influence of age on prevalence and colonization rates in older animals are low.
- The increase in the overall shedding of paired samples was due to an increase on prevalence of feedlot 3, which accounted for 78% of positive samples at exit. The reason why this specific feedlot had an increase in prevalence is uncertain as there were no known management differences. The influence of different management factors on the shedding of C. difficile deserves further investigation.
- The predominance of ribotype 078 was not surprising, and provides further evidence that this is a food animal-associated strain. The clonal nature of C. difficile in this study (and perhaps in cattle in general) complicates molecular epidemiological study.

Conclusions

- The prevalence of C. difficile in beef cattle was low at arrival and at exit from feedlots,
- The significant increase in one lot indicates that studies are needed to look into factors influencing C. difficile shedding.
- Ribotype 078/NAP7 was the only strain present among all isolates recovered.
- It is apparent that C. difficile is carried in the intestinal tracts of a small percentage of beef cattle going to slaughter, but the public health significance requires further study.

Table 1: Prevalence (%) and confidence intervals of the shedding of C. difficile by beef cattle in Alberta, Canada.

<table>
<thead>
<tr>
<th>Feedlot</th>
<th>Overall</th>
<th>Pair Sample</th>
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<tbody>
<tr>
<td></td>
<td>Arrival</td>
<td>Exit</td>
</tr>
<tr>
<td>Feedlot 1</td>
<td>2.4% (3/122)</td>
<td>6.8% (12/171)</td>
</tr>
<tr>
<td>Feedlot 2</td>
<td>7.4% (9/122)</td>
<td>6.8% (9/135)</td>
</tr>
<tr>
<td>Feedlot 3</td>
<td>2.7% (3/102)</td>
<td>11.6% (11/94)</td>
</tr>
<tr>
<td>Feedlot 4</td>
<td>1.7% (3/179)</td>
<td>3.4% (3/88)</td>
</tr>
<tr>
<td>Overall</td>
<td>3.2% (18/543)</td>
<td>5.2% (3/53)</td>
</tr>
</tbody>
</table>

References


Kato H, Kato N, Watanabe K, Iwai N, Nakamura H, Yamamoto T, Suzuki K. (Bidet et al., 1999), If the ribotype pattern was a recognized international ribotype, as determined thorough comparison with a reference strain, it was given the appropriate numerical designation (i.e. 078) Otherwise, an internal nomenclature was used.


Acknowledgements

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