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Clostridium difficile in beef in Australia

Part I

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Contents

1 Background

There is great concern world-wide about a new infectious diseases threat following the recent emergence in Canada,[1] the USA,[2] and now Europe,[3] of a highly virulent strain of Clostridium difficile (called PCR ribotype 027 in Europe and NAP1 in the USA). Rates of detection of C. difficile have risen dramatically, C. difficile disease has been more severe, and attributable mortality was >10% in those aged >60 years.[1] C. difficile is the most commonly diagnosed cause of infectious hospital-acquired diarrhoea in developed countries. The majority of patients with C. difficile-associated diarrhoea (CDAD) have been exposed to antimicrobials that reduce 'colonisation resistance' of the large intestine allowing subsequent infection with C. difficile. Acquisition of C. difficile is facilitated by its ability to form spores that are resistant to many disinfectants allowing it to remain viable in the hospital environment for long periods of time. Toxigenic isolates of C. difficile usually produce two toxins, toxin A and toxin B, and these are thought of as the major virulence factors.[4]

Some strains of C. difficile produce an additional toxin, binary toxin (actin-specific ADP-ribosyltransferase, CDT), first reported in 1988 but not considered important until now.[1,2,5] Binary toxin producers make up the majority of strains isolated in the large outbreaks of disease overseas.[1,2] Barbut et al.[5] showed a correlation between binary toxin production and severity of diarrhoea, and more community-acquired CDAD was caused by binary toxin producers. However, the significance of binary toxin clearly needs further investigation. Although supernatants from $A^-B^-CDT^+$ strains of C. difficile caused fluid accumulation in a rabbit ileal loop after concentration and trypsinisation, challenge of clindamycin-treated hamsters with these strains resulted in colonisation but not diarrhoea or death.[6]

A second important feature of this "new" organism is that it produces more toxin A and B than other strains. Production of these toxins in C. difficile is encoded by the 8.1 kb tcdA and 7.9 kb tcdB genes, respectively. These two genes form part of a highly stable 19.6 kb pathogenicity locus (PaLoc) which also includes tcdC, tcdD and tcdE. Toxin A variant strains fail to produce detectable toxin A by enzyme immunoassay (EIA) because of a deletion in the tcdA gene. The tcdC gene is a putative down regulator of toxin A and B production. The PCR ribotype 027/NAP1 strain has a deletion in the tcdC gene resulting in it no longer down regulating and strains produce toxin throughout log phase of growth instead of just stationary phase.[7] Non-toxigenic strains lack the PaLoc.

The third important feature of these strains is that they are resistant to fluoroquinolone antibiotics, and excessive fluoroquinolone use appears to be a contributing factor in the recent outbreaks.[8] Another significant finding from the outbreaks reported overseas is the marked variation in CDAD rates among different age groups. While the elderly have always been at increased risk of CDAD, due primarily to decreased host defences, rates in persons ≥65 years of age have increased dramatically since 2000.[9] One possible novel risk factor is exposure to gastric acid suppressants such as histamine-2 receptor inhibitors or proton pump inhibitors. These agents have been more commonly prescribed in recent years and may be associated with increased rates of CDAD in the community,[10] although some case-control studies with hospital patients show no association.[1,8] The importance of community onset CDAD was highlighted recently by a report of severe CDAD in previously healthy persons and peripartum women.[11]

The new quinolone antimicrobials have significantly better anti-anaerobe activity than ciprofloxacin and are likely therefore to have a greater impact on colonisation resistance.[12] As mentioned above, it is possible that this issue, as well as increasing resistance of C.difficile

strains to the quinolones, is contributing to the significant increase in C. difficile diarrhoea worldwide.[13] The recent reports from Canada and the USA suggest that a strain of C. difficile has emerged that is both resistant to quinolones and a hyper-producer of toxins A and B, as well as producing binary toxin.[2,14]

One possible source of C. difficile in the community is animals. C. difficile has been associated with enteric disease in a variety of animals, including horses, pigs, cats and dogs.[15-17] Although it is not yet completely clear, it is possible that in all these situations excessive antibiotic exposure is driving the establishment of C. difficile in animals, in a manner analogous to human infection, rather than the organism just being normal flora of the animal gastrointestinal tract. Of great significance to Meat & Livestock Australia (MLA) are two recent reports that C. difficile, including the epidemic ribotype 027, has been isolated from both calves[18] and retail meat samples[19] in Canada. C. difficile was isolated from 20% of 60 retail meat samples collected over a 10 month period in 2005. Clearly these meat samples were contaminated by C. difficile present in the bovine gastrointestinal tract. What risk such contamination poses for food-borne transmission of C. difficile, and the role of antibiotics in animal carriage of C. difficile, is unknown.

Currently, there are no data on the prevalence of C. difficile carriage in Australian cattle. What risk such contamination poses for food-borne transmission of C. difficile is unknown. To get a better estimation of the risk in Australia it would be logical as a first step to undertake a survey of Australian cattle going to slaughter.

2 Study aims

1. To undertake a survey of Australian cattle for the presence of *C. difficile*, and determine the prevalence and concentration.
2. *C. difficile* isolates recovered would be typed to see if there is any relationship with humans isolates in Australia.
3. Assess any risk of food-borne transmission of *C. difficile* from contamination.

3 Methods

Bacteria

An isolate of *C. difficile* PCR ribotype 027 was obtained from Dr Luis Arroya at the University of Guelph, Canada. This and a fluoroquinolone resistant local isolate of *C. difficile* (WA15) were used as controls.

Specimens

Samples of adult cattle gastrointestinal contents (approx. 50 g) and carcass washings (approx. 50 ml) were collected on six occasions from an abattoir in Western Australia. They were transported to Perth the same day, stored at 5ºC and processed within 24 hours.

Culture for *C. difficile*

Attempts to isolate *C. difficile* were made based on our previously described methods[20] with some modifications. Intestinal contents were cultured both directly on CCFA and in an enrichment broth, while carcass washings were centrifuged and the deposit inoculated into an enrichment broth. All plates were incubated in an anaerobic chamber (Don Whitley Scientific

Ltd.) at 37°C, in an atmosphere containing 80% nitrogen, 10% hydrogen and 10% carbon dioxide. Three control strains were used to monitor anaerobiosis; *P. aeruginosa* ATCC 27853, *C. difficile* ATCC 43593, and *M. luteus* ATCC 4698. After 48 hours incubation, all enrichment broths were sub-cultured onto CCFA and incubated as above.

Identification of *C. difficile*

C. difficile was identified on the basis of characteristic colony morphology (yellow, ground glass appearance) and odour (horse dung smell). The identity of doubtful isolates was confirmed by Gram stain and a latex agglutination test kit (Oxoid).[21]

Toxin gene B PCR assay
Gastrointestinal contents were tested for the presence of toxin B gene DNA by a PCR assay, based on that previously described by Kato *et al*.[22] One fragment from the non-repeating region of toxin B was amplified by real time PCR.

4 Results

1) Gastrointestinal contents

A total of 158 samples of gastrointestinal contents was processed. *C. difficile* was not isolated from any sample, either on direct culture or by enrichment culture.

2) Carcass washings

A total of 151 samples of carcass washings was processed. *C. difficile* was not isolated from any sample by enrichment culture.

3) Toxin gene B PCR assay

All toxin B gene PCR assays were negative.

5 Conclusions

Neither viable *C. difficile* nor *C. difficile* DNA was detected in any sample, suggesting that *C. difficile* was not present in cattle in Australia, and therefore posed no risk to consumers.
However, these results should be interpreted with some caution. While a promising first look at the situation in cattle in Australia, there are some limitations to the study. First, only one abattoir in one geographic location in Australia was investigated. There may be some geographical variation in the distribution of *C. difficile* based on antibiotic prescribing practices and/or the use of growth promotants. The use of antibiotics in cattle was not investigated but may serve as a potential lead for the presence of *C. difficile*.
Second, only adult cattle were investigated. There is a growing body of evidence that many neonatal or infant animals are colonized with *C. difficile*, including cattle.[23] Whether such colonization continues beyond the infant period may well depend on exposure to antimicrobials.

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