**Clostridium difficile** in Food and Domestic Animals: A New Foodborne Pathogen?

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(See the editorial commentary by Rupnik, on pages 583–584.)

**Clostridium difficile** infection is increasingly recognized as a cause of diarrhea in outpatients and persons with no apparent health care facility contacts. In contrast to **C. difficile** infection acquired in health care settings, few risk factors for development of community-associated **C. difficile** infection are known. Foodborne transmission of **C. difficile** has been hypothesized as a possible source for community-associated infections; however, the evidence to confirm or refute this hypothesis is incomplete. Recent studies have demonstrated isolation of **C. difficile** from foods in the United States, Canada, and Europe and from meat products intended for consumption by pets. This raises questions about foodborne transmission of this pathogen to humans through consumption of contaminated products. This review summarizes the available data on **C. difficile** in animals and food and discusses the potential for foodborne transmission of this pathogen.

**Clostridium difficile** is a gram-positive spore-forming anaerobic bacterium that causes disease in humans and animals ranging from asymptomatic colonization to diarrhea and colitis. Infection may be life threatening. Although **C. difficile** infection (CDI) has typically been associated with exposure to health care settings, especially among patients who have taken antibiotics, CDI is increasingly recognized as a cause of diarrhea in outpatients and persons with no apparent health care contacts [1, 2]. Community-associated infections have been described in populations traditionally considered to be low risk, including the young and those without antibiotic exposures [1, 3, 4]. Other risk factors for development of community-associated CDI have not been demonstrated, leaving unanswered the question of how transmission occurs in the community.

Food has been hypothesized as a possible source of **C. difficile** in community settings, but evidence to confirm or refute this hypothesis is incomplete. **C. difficile** is recognized as both a gut colonizer and cause of diarrhea in food animals [5–9]. Recent studies have isolated **C. difficile** from retail foods intended for human consumption in the United States [10], Canada [11–13], and Europe [14–16] and from meat products intended for consumption by pets [17, 18]. These findings support concerns about foodborne acquisition of this pathogen through consumption or handling of contaminated products; however, no published studies have documented consumption of any food product as a risk factor for CDI. An improved understanding of the relationship between animal and human strains of **C. difficile** will help to evaluate the potential for foodborne transmission and the role of animal-human contacts in **C. difficile** epidemiology. We summarize the available data on **C. difficile** in animals and food and discuss data gaps that must be addressed to clarify whether foodborne transmission of this pathogen might occur, and if so, whether this route might be important in the epidemiology of CDI.

**STRAIN TYPING CONSIDERATIONS**

One factor complicating our understanding of the extent of common **C. difficile** strains between animals and humans is the lack of a standard nomenclature and typing system used routinely by different research groups. Results of polymerase chain reaction (PCR)-ribotyping [19], used routinely in Europe, are not directly comparable between laboratories and require that a laboratory have reference isolates for comparison to assign a standardized type that is meaningful across laboratories [20–22]. Restriction endonuclease analysis (REA) displays excellent discriminatory power [19] but has limitations similar to PCR-ribotyping with regard to interlaboratory comparison and is
not widely used. Results of pulsed-field gel electrophoresis (PFGE), routinely used by reference laboratories in the United States and Canada, are more portable but may have limited specificity in nonoutbreak settings [21]. Toxinotyping, which targets variations in the C. difficile pathogenicity locus, is reproducible and portable between laboratories but is less discriminatory than other methods and is not often used as a stand-alone method. Although there is generally good concordance among the different typing methods, strain information obtained with one typing method may not correctly infer the strain type obtained by an alternate typing method. Thus, much of the data regarding C. difficile strain prevalence cannot be generalized without a specific additional effort to type a given strain by multiple methods, and thus, many researchers publish using internally generated nomenclature, which cannot be generalized.

**C. DIFFICILE IN DOMESTIC ANIMALS**

C. difficile is both a commensal organism and a pathogen in domestic and food animals, but not all studies have compared animal isolates with those known to cause human illness. Although an early study found no relationship between isolates recovered from cats and dogs and those from humans [23], recent studies found considerable overlap among bovine, equine, porcine, canine, and human isolates [9, 24]. In all of these studies, certain strains appeared to be largely species specific, whereas others were found across multiple species, including humans. Of particular note, in studies from several countries certain strains have been indistinguishable between humans and other mammals [25–27], suggesting a common source, human-to-animal transmission, or zoonotic transmission.

In particular, toxinotype (TOX) V/PCR ribotype 078/PFGE type NAP7 or NAP8/REA type BK strains have been increasingly identified as predominant strains in cattle and pigs in the United States [9] and Europe [9, 27] and also as important pathogens in humans [25, 28]. In the Netherlands, 4%–11% of humans with CDI carried ribotype 078 [27], and from February 2005 through February 2008 the incidence of infection with this strain increased 4-fold, making it the second most common ribotype isolated from humans in that country [28]. Unlike the geographic distribution of the current epidemic strain, NAP1/027/BI, which mirrored population density in the Netherlands, the distribution of human infections with ribotype 078 was concentrated in more rural areas where pigs are raised [28]. Ribotype 078 isolates from pigs and humans in the Netherlands were indistinguishable by PCR ribotype and by multilocus variable-number tandem repeat analysis, a more discriminatory strain typing approach [26]. In the United States, human infections caused by TOX V/NAP7 or NAP8/REA type BK strains have also increased relative to other strains and may be more common among community-associated CDI than health care–associated CDI [25]. Additionally, several NAP7/078/TOX V isolates from food animals from the United States were found to be very closely related to or indistinguishable from human isolates by PFGE [25].

The current epidemic strain, NAP1/027/BI, has caused outbreaks of human disease in North America and Europe for nearly a decade and is a cause of sporadic disease worldwide [29, 30]. The emergence of NAP1/027/BI in North America corresponds with increases in both incidence and severity of health care–associated CDI and may have been driven largely by use of fluoroquinolone antibiotics [29]. The recognition that community-associated CDI accounts for a substantial proportion (~20%) of all CDI in North America [31–33] also occurred at the same time of the emergence of the NAP1/027/BI strain, suggesting that NAP1/027/BI has affected the epidemiology of both community-associated and health care–associated CDI.

NAP1/027/BI has been isolated from both food and companion animals. In Canada, ribotype 027 was the third most common type isolated from calves [8] and was found from widely geographically dispersed sites. Ribotype 027 has also been isolated a horse [34] and a hospital visitation dog in Canada, which likely acquired its infection during a visit to a health care facility experiencing an outbreak with the same 027 strain [35].

Other C. difficile strain types known to cause disease in humans, including ribotype 017 (TOX VIII/NAP9) and ribotype 066 [7], have also been isolated from animals [8, 36, 37]. C. difficile has also been isolated from household pets, including cats and dogs, and their environment [23, 35, 38, 39].

Clearly, C. difficile is an established human and animal pathogen and there is considerable overlap among some animal and human strains. It is unclear, however, what this implies about the epidemiology of CDI, specifically, whether and how zoonotic transmission occurs. No study has demonstrated acquisition of a human C. difficile infection as a result of animal contact, although animal acquisition of C. difficile from humans has been suggested by some studies [35, 40]. Even if transmission of C. difficile from animals to humans occurs rarely, such contact may be an important source of new strains of C. difficile among humans, including antibiotic resistant strains. Antibiotic resistance, including fluoroquinolone resistance, is a common trait of many C. difficile strains that have recently emerged in humans [41]. More epidemiologic studies are needed to determine the role of animal contact in transmission of C. difficile to humans and vice versa and whether animal-to-human transmission occurs, to document the risks attributable to this mode of transmission.

**C. DIFFICILE IN RETAIL FOOD PRODUCTS**

The first study to report isolation of C. difficile from retail meat, conducted over 10 months during 2005 in 2 Canadian prov-
Table. 1. Summary of Findings from Studies Demonstrating the Presence of Clostridium difficile in Retail Foods

<table>
<thead>
<tr>
<th>Country (region), product</th>
<th>No. of positive samples/total no. cultured (%)</th>
<th>PCR ribotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States (Arizona)</td>
<td></td>
<td></td>
<td>[10]</td>
</tr>
<tr>
<td>Ground beef</td>
<td>13/26 (50.0)</td>
<td>027, 078</td>
<td></td>
</tr>
<tr>
<td>Summer sausage</td>
<td>1/7 (14.3)</td>
<td>027</td>
<td></td>
</tr>
<tr>
<td>Ground pork</td>
<td>3/7 (42.9)</td>
<td>027, 078</td>
<td></td>
</tr>
<tr>
<td>Braunschweiger</td>
<td>10/16 (62.5)</td>
<td>027, 078</td>
<td></td>
</tr>
<tr>
<td>Chorizo</td>
<td>3/10 (30)</td>
<td>027, 078</td>
<td></td>
</tr>
<tr>
<td>Pork sausage</td>
<td>3/13 (23.1)</td>
<td>027, 078</td>
<td></td>
</tr>
<tr>
<td>Ground turkey</td>
<td>4/9 (44.4)</td>
<td>078</td>
<td></td>
</tr>
<tr>
<td>Canada (Ontario, Quebec)</td>
<td></td>
<td></td>
<td>[11]</td>
</tr>
<tr>
<td>Ground beef</td>
<td>11/53 (20.8)</td>
<td>077, M31, 014, M26</td>
<td></td>
</tr>
<tr>
<td>Ground veal</td>
<td>1/7 (14.3)</td>
<td>M31</td>
<td></td>
</tr>
<tr>
<td>Canada (nationwide)</td>
<td></td>
<td></td>
<td>[12]</td>
</tr>
<tr>
<td>Ground beef</td>
<td>10/149 (6.7)</td>
<td>M26, 077, J, 014, C, F, H</td>
<td></td>
</tr>
<tr>
<td>Veal chops</td>
<td>3/65 (4.6)</td>
<td>M26, J, K</td>
<td></td>
</tr>
<tr>
<td>Canada (British Columbia, Saskatchewan, Ontario, Quebec)</td>
<td></td>
<td></td>
<td>[13]</td>
</tr>
<tr>
<td>Ground beef</td>
<td>14/115 (12.2)</td>
<td>078, 027, C</td>
<td></td>
</tr>
<tr>
<td>Ground pork</td>
<td>14/115 (12.2)</td>
<td>078, 027, C, E, Y</td>
<td></td>
</tr>
<tr>
<td>Scotland</td>
<td></td>
<td></td>
<td>[14]</td>
</tr>
<tr>
<td>Salad</td>
<td>3/40 (7.5)</td>
<td>017, 001</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. PCR, polymerase chain reaction.

inces, reported C. difficile isolation from 20% of retail meat products sampled, including 21% of ground beef and 14% of ground veal samples [11]. In a follow-up study conducted using an expanded national random sampling scheme [12], the overall prevalence of C. difficile in Canadian retail beef products was 6.1% (6.7% in ground beef and 4.6% in veal chops). Prevalence varied by season, with the highest prevalence (11.5%) observed during January and February. Nearly all of the isolates recovered were toxigenic, and most were related to strains associated with human infection, including several that were TOX III and identified as NAP1 or NAP1-related by PFGE [11, 12]. Isolates belonging to other strains known to infect humans were also identified, including ribotype 077/TOX 0/(NAP4), isolated from cattle, dogs, and humans [42], and ribotype 014/TOX 0/(NAP4 or NAP6) [37], isolated from cattle and humans [42, 43]. A common nontoxigenic strain, classified as PCR ribotype M26, that has also been isolated from dogs, was recovered from retail meat products in both studies [11, 12, 24].

In a study of more varied retail meat products conducted in the United States, C. difficile was isolated from 42% of retail beef, pork, and turkey samples purchased at grocery stores in Tucson, Arizona over 3 months [10]. Although all of the products were purchased in the same city, the products were, with 1 exception, nationally distributed brands. C. difficile was found in both raw and ready-to-eat products and in meats from all animal origins tested. The proportion of positive samples varied little by type of meat product (Table 1). Most of the isolates (73%) recovered were ribotype 078/TOX V/NAP7 or NAP8 and were similar to those obtained from food animals and humans. The remaining isolates were ribotype 027/TOX III/NAP1 or NAP1 related.

In another study, C. difficile was isolated from 3 (7.5%) of 40 ready-to-eat retail salads by researchers in Scotland [14]. The salads from which C. difficile was isolated were imported from European Union countries and did not originate from United Kingdom suppliers. All 3 C. difficile isolates were toxigenic, including 2 ribotype 017 (TOX VIII/NAP9) isolates, a common strain causing CDI in Europe [44], and 1 ribotype 001 (TOX 0/NAP1) isolate, common among clinical isolates in the United Kingdom and a previous epidemic strain in the United States [20]. C. difficile has also been isolated from retail meats in Europe, but the rate of contamination appears to be lower than that reported in the United States and Canada [15, 16, 45].

Variation in culture methods might contribute to the apparent variation in C. difficile prevalence in different studies of retail meat. There are currently no published data addressing the best methods for detection of C. difficile in foods. Although these studies used varied culture methods, each included a broth enrichment step, which makes it difficult to quantitatively assess the burden of C. difficile spores in foods. However, a recent study of retail ground beef and ground pork in Canada suggests that spore burden is low and that C. difficile is more reliably detected by protocols that include an enrichment step than by direct plating alone [15]. In this study, a direct plating method detected <30% of C. difficile–containing samples;
products are newly available, and there are limited epidemiologic data to connect \textit{C. difficile} found in the food supply to human illness. However, the epidemiology of \textit{C. difficile} infection is changing, including an increase in both incidence and severity of disease, emergence of a new epidemic strain (ribotype 027/NAP1), and an apparent increase in infections among persons in community settings. Increasing rates of CDI in the community have raised questions about origins of new human strains, sources of human \textit{C. difficile} acquisition, and risk factors for the development of infection. In addition to causing human disease, CDI is recognized as a cause of epidemic disease in piglets [5], and \textit{C. difficile} is also commonly found in other food animals, including cattle and chickens [8, 47–49]. Some of the \textit{C. difficile} strains most commonly identified in food animals appear to be emerging as causes of disease in humans, especially among humans with community-associated CDI [25]. Although a link between \textit{C. difficile} carriage in animals and disease in humans has not been adequately defined, some investigators have suggested that food animals may play an important role in the expansion of pathogenic \textit{C. difficile} clones and in transmission to humans through food [50]. The presence of \textit{C. difficile} in national brands of retail meats and in both the United States and Canada suggests that exposure to \textit{C. difficile}–contaminated foods may not be limited to any geographic region or regional food animal processing facilities. Additionally, the presence of spores in several types of products, as well as the isolation of indistinguishable strains from foods of different animal species origin, suggests that contamination may affect a range of retail food products. The finding of potentially infectious spores of an important human pathogen in retail food products is provocative, particularly because detection of \textit{C. difficile} multiple retail food types from numerous countries and geographic regions [10–14] suggests that exposure of humans via food may be a common occurrence.

If transmission indeed occurs from animals to humans, it will be essential to characterize the dynamics of this transmission, including whether transmission occurs through direct animal-to-human contact or through indirect means, such as consumption of contaminated foods. Increasingly, foods such as produce have been recognized as vehicles for pathogen transmission in outbreaks [51]. In many of these outbreaks, a contaminated environment (eg, soil or irrigation water) appears to be responsible for delivery of bacteria to the food plants. In some instances, pathogens are internalized by the plant during growth, limiting the efficacy of control measures based on sanitation or washing [51]. \textit{C. difficile} has also been isolated from produce [14, 52] and can be recovered from a wide variety of environmental sources, including soil, sea water, and fresh water [52]. Thus, it is possible that humans and animals are frequently exposed to \textit{C. difficile} spores from multiple sources. Whether, when, and how frequently this exposure leads to disease is a critical question for improved control of CDI.

**FUTURE RESEARCH NEEDS**

A number of important questions must be answered to determine whether foodborne transmission of \textit{C. difficile} occurs and to determine the possible impact of low-level spore contamination on the safety of the food supply. For example, the infectious dose of \textit{C. difficile} for humans is unknown; if the infectious dose were known, it could be compared with the microbial burden that is typically present on contaminated foods at the point of consumption. Infectious dose is likely to vary depending on host factors, including age, underlying medical conditions, and exposure to antibiotics and acid-reducing medications, and these factors are likely to be very different between hospitalized and community populations. \textit{C. difficile} is not considered to be part of the normal human intestinal flora, but limited studies have demonstrated presence of toxigenic \textit{C. difficile} in 3%–5% of asymptomatic persons in the community [53]. It is unknown whether this finding represents subclinical infection, colonization, or transient pass-through of ingested spores.

It is also unknown whether or how often \textit{C. difficile} is trans-
mitted from animals to humans, or vice versa, or whether presence of common strains in animals and humans reflects exposure to a common environmental reservoir. Surveillance for human and animal infections is needed and should include subtyping studies designed to distinguish between common sources of animal and human infection or animal-to-human transmission. Detailed strain typing and epidemiologic investigations designed to evaluate the role of foodborne transmission during C. difficile outbreaks might help to determine whether C. difficile strains found in humans are linked to the food supply. Additionally, studies are needed to characterize food and environmental exposures in persons with community-associated CDI who do not have any health care exposures and to clarify whether implicated risk factors also impact transmission in health care settings.

Studies are needed to develop consensus best-practice methods to test meats and other foods for C. difficile. In addition, more work is needed to understand the effect of heating and surface decontamination on C. difficile spores in and on meat and other food products and, if foodborne transmission proves to be a mechanism, to evaluate other possible approaches to limit transmission by this route.

It is reasonable to assume that the general public is and has been often exposed to low numbers of potentially infectious C. difficile spores. There is currently limited epidemiologic evidence to support or refute the hypothesis that C. difficile is transmitted by the foodborne route; the presence of C. difficile on retail foods suggests but does not prove that some proportion of infections is acquired this way. The food supply may thus serve as a source of new strains causing human infections; alternatively, food could be another constant and normally innocuous exposure. It is very clear that more research is needed to better understand the dynamics of and risk factors for development of CDI among persons in the community, including the relevance and possible importance of foodborne transmission.

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