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NOTES

Prevalence of PCR Ribotypes among *Clostridium difficile* Isolates from Pigs, Calves, and Other Species[▽]

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PCR ribotypes were obtained for 144 *Clostridium difficile* isolates from neonatal pigs. Porcine isolates comprised four PCR ribotypes, but one, ribotype 078, predominated (83%). This was also the most common ribotype (94%) among 33 calf isolates but was rarely identified in other species.

Numerous epidemiologic investigations of human *Clostridium difficile*-associated disease (CDAD) have employed phenotyping and genotyping to better understand transmission patterns and risk factors (3, 7, 8). In contrast, few studies have typed isolates from animals or environments where they are housed. Most studies attempted to test the hypothesis that humans acquire *C. difficile* from animals, and the results have varied (1, 9). We know little about the epidemiology of *C. difficile* within animal populations, and uncertainties persist as to the relationships, if any, of such animal strains to those associated with human CDAD.

CDAD has emerged as a significant economic concern for swine producers, but little is known about transmission patterns in hog operations (11, 14). The objective of this study was to assess the strain types occurring among various species with CDAD. PCR ribotyping was used due to its proven discriminatory power, reproducibility, and relative simplicity (2). Different strains of *C. difficile* possess different numbers of rRNA genes, and the intergenic sequences vary in size, yielding PCR products of distinct sizes for each designated ribotype. Isolates from five host species were examined, but porcine isolates were the most numerous (Table 1). Neonatal pigs in Iowa, Ohio, Montana, North Carolina, and Utah were sampled. All bovine isolates were collected from male dairy calves (<2 months of age) shipped to Arizona from multiple states (the precise origins were not available). Equine isolates were from Kansas ($n = 1$), Arizona ($n = 1$), and Ontario, Canada ($n = 17$), or an unknown source ($n = 1$). Canine isolates were from Ontario. Human isolates were from patients in two hospitals in Colorado and one hospital in Louisiana. Ages of dogs, horses, and humans were typically not reported.

Primary isolations were made by culturing stool samples or rectal swabs by using standard techniques (5, 15). Colonies were passed through brain heart infusion agar with cysteine (0.05%), yeast extract (0.5%), and defibrinated bovine blood (2%). All isolates were examined for the toxin genes *tcdA* and *tcdB* by use of an established multiplex PCR technique (6, 13). PCR ribotyping was performed as previously described (10, 12). Ribotype classifications were based on the typing scheme established at the United Kingdom Anaerobe Reference Unit in Cardiff, Wales (12).

Nineteen PCR ribotypes were identified from the 232 *C. difficile* isolates examined (Table 1). Bovine and swine isolates were much less diverse than those from dogs, horses, or humans. Three PCR ribotypes were identified from calves, and four were identified from pigs. PCR ribotype 078 (type 078) accounted for 94% of bovine and 83% of swine isolates. This ribotype did not occur among the canine isolates and represented only 5.0% of equine and 4.4% of human isolates.

Canine isolates comprised five ribotypes, and 42% were type 010. Most equine isolates (30%) were type 015. Human isolates included 12 ribotypes. The most common was type 020, with 22% prevalence. Overall, there was a considerable overlap of equine and human ribotypes with those of other species (Table 1). However, 4 of the 12 human-associated ribotypes did not occur in any of the other host species.

Although more isolates from pigs and calves were examined, much less ribotype diversity was observed among these species. The limited diversity among porcine isolates is even more remarkable when we consider that they were collected from a large geographic area. In contrast, the origins of canine, equine, and human isolates lacked significant geographic variability.

Type 078 was predominant in both pigs and calves, although it is almost certain that there was no interspecies contact. The swine operations that were the source of isolates maintained strict biosecurity such that pigs at each location were isolated not only from other species but also from other herds of pigs. Biosecurity associated with the calves was much more relaxed. Nonetheless, dairy calves were typically kept in confinement

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TABLE 1. *C. difficile* PCR ribotype prevalence by species

Ribotype	No. of isolates (% of total)				
	Bovine	Canine	Equine	Swine	Human
001			1 (5)		3 (13)
002	1 (3)		3 (15)	6 (4)	2 (9)
009		2 (17)	3 (15)		
010		5 (42)	2 (10)		3 (13)
012			1 (5)		1 (4)
015		1 (8)	6 (30)		1 (4)
017					1 (4)
020		2 (17)	1 (5)		5 (22)
031					
033	1 (3)			3 (2)	
056					2 (9)
077		2 (17)			
078	31 (94)		1 (5)	119 (83)	1 (4)
087					
103					1 (4)
126				16 (11)	1 (4)
137			1 (5)		
151					2 (9)
154			1 (5)		
Other					
Total	33	12	20	144	23

with no exposure to other species. The only factor common to both species is close contact with human caretakers. However, only one of the human isolates was type 078. This ribotype did not occur among the 13 canine isolates and was represented by only one of the equine isolates. Therefore, it appears that type 078 is the most common PCR ribotype circulating independently among pigs and calves in the United States and among numerous widely separated populations of pigs. An alternative hypothesis is that pigs and calves are exposed to varied *C. difficile* strains but are both more susceptible to colonization by type 078. It seems unlikely that such different species would share a common susceptibility not seen in the other species examined, but this hypothesis cannot be disproved by our data alone.

Age-specific physiology should also be considered. The calves were functional monogastrics due to their age and, like the pigs, subsisted on milk. Milk, even from two different species, is more similar than the feed rations given to adult hogs and cows. Furthermore, the microbial environment of the two species, as adults, undoubtedly contributes to a great disparity in the normal flora and microbial food chain. It would be interesting to compare ribotypes of *C. difficile* isolates from adult cows and hogs to those occurring in the neonates.

Even though a single ribotype was predominant in the majority of pigs and calves, it is a mistake to assume that there is no heterogeneity among these isolates. Differences in colony morphology among isolates of type 078 from both species were noted (data not shown) and may reflect some underlying strain differences. PCR ribotyping is a useful technique, but its discriminatory power is not absolute. Type 001, a common ribotype causing human CDAD, can be subtyped using other methods including randomly amplified polymorphic DNA, ribospacer PCR, and pulsed-field gel electrophoresis (4). It cannot be assumed that isolates of type 078 from pigs and calves are identical, but this technique does demonstrate that they differ significantly from the most common ribotypes occurring in all other species examined.

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