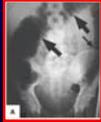


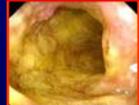
Clostridium difficile: An emerging zoonosis?

W. A. Gebreyes, DVM, PhD., DACVPM
The Ohio State University
College of Veterinary Medicine
Department of Veterinary Preventive Medicine



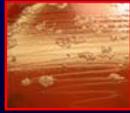
Background

- *Clostridium difficile*
 - a Gram-positive, spore forming anaerobe
 - humans, animals, and the environment.
- Most commonly diagnosed cause of antimicrobial- and hospital-associated diarrhea-
 - *C. difficile* Associated Diarrhea (CDAD)
- The only known cause of antimicrobial-associated pseudomembranous colitis. (Fordtram, 2006)



C.difficile in Household Pets

- Household pets have been implicated as a potential reservoir of *C. difficile*
 - Feces of clinically normal and diarrheic dogs
 - Carriage rates of both toxigenic and non-toxigenic *C. difficile*
 - ranging from 0% to 10% in healthy dogs in the community.
 - The most common toxigenic ribotype, which accounted for 90% of toxigenic isolates, is also a recognized cause of CDAD in humans (Arroyo et al., 2004).



C. difficile in Horses

- Important cause of colitis in adult horses and foals
- Primarily due to treatment with antimicrobials during hospital stays
- Isolated from healthy foals and the environment
 - May serve as reservoirs of toxigenic strains
- Important cause of duodenitis-proximal jejunitis (DJ)
 - Found significantly more in horses suffering from DJs compared to controls (Weese 2006)



C. difficile in Cattle

- A retrospective study
 - a strong association between the presence of fecal *C. difficile* toxins and calf diarrhea (Rodriguez-Palacios et al 2006)
- Calves- multiplying hosts of the organism (Songer 2008)
- *C. difficile*- isolated from retail ground beef
 - intended for human consumption (Rodriguez-Palacios et al 2007)



C. difficile in Swine

- Important cause of neonatal enteritis in pigs
- Pigs between 1-7 days age develop CDAD
 - characterized by edema of the colon and feces are watery in consistency
- More than two-third of the litter can be positive for toxin when infected by *C. difficile* (Waters et al., 1998; Yaeger et al., 2002).
- Loss of weight- between 10-15% (Songer and Uzal, 2005)
- It has been postulated
 - swine and associated products are responsible for the community-acquired C. difficile strains in humans.



Human CDAD and risk factors

- The traditional risk factors
 - Old age
 - Antimicrobial therapy
 - High gastric pH (*Fordtran, 2006*)
- Others: Contact with infected patients, contact health care providers and weak immune system

Table 2. Correlation of pH and acid concentration in gastric juice*

pH	[H ⁺] mEq/L	Effect on <i>C. difficile</i> spore germination
1	100	Killed
2	10	
3	1	
4	0.1	Survive
5	0.01	
6	0.001	
7	0.0001	

*The negative base of Clostridium difficile spores exposed to gastric juice at a pH of 1 to 3, had the spore count after gastric juice pH 4 or 5 above 10⁶. The spores of *C. difficile* are not killed by gastric acid.
 †Indicates higher concentration.



Changes in Epidemiology of *C. difficile*

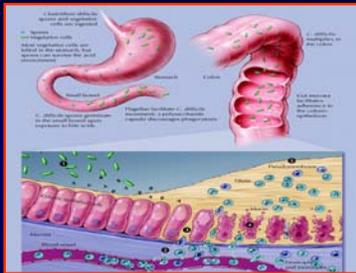
- Recently, the **incidence and severity of disease has increased as well as the frequency in low-risk populations.**
 - Young people
 - No prior antibiotic therapy
 - No exposure to health-care settings (*Chernakl et al., 2005*)

TABLE 1. Clinical features of *Clostridium difficile*-associated disease (CDAD) in patients* with community and peripartum exposures, by case type and selected characteristics — New Hampshire, New Jersey, Ohio, and Pennsylvania, 2005

Type	Characteristic													
	Aged ≤ 18 yrs	Female sex	Previous antimicrobial use [†]	Contact with gastrointestinal illness [‡]	Bloody diarrhea	Hospitalization necessary for CDAD treatment	Emergency department visit necessary	Relapse						
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)				
Community	1123	(48)	1523	(65)	1523	(65)	723	(30)	623	(26)	323	(13)	823	(35)
Peripartum	910	(0)	1010	(100)	910	(90)	910	(9)	210	(20)	410	(40)	210	(20)
Total	1193	(33)	2533	(78)	2433	(72)	723	(30)	833	(24)	1033	(30)	533	(15)

*N = 83.
 †Defined as receipt of an antimicrobial within 3 months before diarrhea onset.
 ‡Defined as direct or household contact with another person with diarrhea illness.

CDAD Pathogenesis and Hypervirulence



Pontanen and Simor, CMAJ, 171 (1), 2004

Two pre-requisites for *C. difficile* infection:

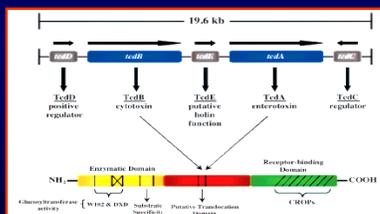
- Damage to the microflora- caused by antimicrobial use
- Ingestion of the pathogen

C. Difficile Toxins

- Toxin A (Enterotoxin):** More critical role in pathogenesis
 - Associated with extensive tissue damage
 - Responsible for GI wall damage
- Toxin B (Cytotoxin):** Elicits effect after Toxin A activity
 - Toxin B is more potent cytotoxin.
- Binary Toxins (*cdtA* and *cdtB*)** are also produced by some strains
 - Role in hypervirulence- not well known

Pathogenicity Locus (PaLoc)

- Chromosomal location
- Composed of five genes
 - tcdA*, *tcdB*, *tcdC*, *tcdD* and *tcdE*



Voth and Ballard, CMR, 18, 2005

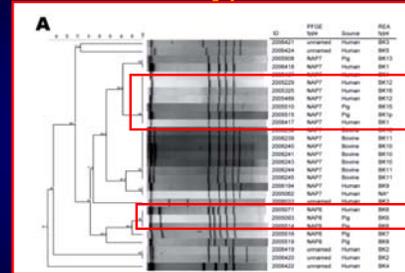
Major Hypervirulent Strains

- NAP1/027/Toxinotype III**
 - Most common (16%) type found hospital outbreaks (*Kuijper 2007*)
 - Binary toxin positive
 - 18bp deletion in *tcdC*
 - Resistant to gatifloxacin and moxifloxacin
- 078/Toxinotype V**
 - 1.3% (USA) to 7.5% (Netherlands) of CDAD cases
 - 83% of swine and 94% of calf isolates (*Songer 2007*)
 - Binary toxin positive
 - 39bp deletion in *tcdC*

Geno- and Toxinotyping

- Pulsed Field Gel Electrophoresis (PFGE)
- PCR-RFLP of the PaLoc region.
 - Profiles are then compared to the parent strain VPI 10463.
 - Differences in fragment pattern of the PaLoc area determine the type
 - At least 20 Toxinotypes (I-XX) have been described so far (Gerig et al., JMM, 53; 2004)
 - **Toxinotype III**- implicated in several outbreaks (McDonald LC et al, 2005).
 - **Toxinotype V** - possibly connected to interspecies transmission (Limbago 2008)

078/Toxinotype V Strain



Analysis of toxinotype V *Clostridium difficile* human and animal isolates using PFGE *Sma*I.

- Three animal-human isolate groups have indistinguishable patterns (McDonald et al, 2008).

Antimicrobial Resistance

- Fluroquinolone resistance- common in outbreak strains (McDonald et al, 2005)
- Resistance to erythromycin, clindomycin, and moxifloxacin have been found more often in recent strains (Schmidt 2007)
- Metronidazole and Vancomycin are the two drugs of choice for treating CDAD
- Recent reports from humans
 - increased frequency of resistance to metronidazole and increase in MIC against vancomycin (Bishara et al, 2006; Pelaez et al 2005)

Recent news in Europe...

- *C. difficile* outranks MRSA in UK
- Deaths involving *C. difficile* rose by 69% to 3,800 from 2004-05 (Office for National Statistics)
- In the same period, MRSA mentions on certificates increased by 39% to 1,629
- Most of the deaths were in the old age group

C. Difficile in Pig and humans: OH and NC

(Gebreyes WA, Fry P, Thakur S, Kaye K, Woods C, Bannerman T)

Hypothesis: **Pigs are source hypervirulent strains, associated with community-acquired infections in humans.**

- Determine the occurrence and prevalence of the hypervirulent strain.
- To determine whether *C. difficile* is able to persist from the farm (farrow to finish) to the food product
- To characterize the various toxin genes associated with hypervirulence
- compare clonality of strains from humans and pigs using toxinotyping and genotyping (PFGE).

Sample Collection

- **Swine**
 - 10 farms (7 from NC and 3 from OH) selected.
 - Fecal samples collected at farrowing, nursery and finishing farms
 - 30 pigs/farm followed prospectively
 - Piglets in litters found to be positive for *C. difficile* tagged and followed for subsequent sample collections at different stages
 - Slaughter and retail meat samples
- **Human**
 - Retrieve isolates or positive stool samples :
 - Local hospitals: Duke Univ./ VA Medical Center
 - ODH

Bacteriology and antimicrobial susceptibility

- Standard bacteriological methods
- Eight antimicrobial panel using Epsilometric (E-test)
 - Ciprofloxacin
 - Erythromycin
 - Metronidazole
 - Gatifloxacin
 - Vancomycin
 - Tetracycline
 - Ampicillin
 - Mobifloxacin

C. difficile Toxino- and Genotyping

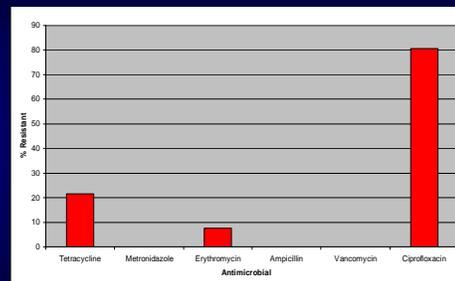
- Toxins A, B (*tcdA*, *tcdB*) and binary toxin (*cdtA* and *cdtB*) detection using PCR
- Deletion in *tcdC* gene (18/ 39 bp or other) using PCR (Warny et al., 2005).
- Genotyping of *C. difficile* isolates using PFGE and toxinotyping
 - Comparison with *C. difficile* isolates from humans

Prevalence

FARROWING			
	Pigs Sampled	Positive	Prevalence
North Carolina-1	30	18	60
North Carolina-2	29	17	58.6
North Carolina-3	32	21	65.6
North Carolina-4	32	28	87.5
North Carolina-5	32	18	56.3
Subtotal	155	102	65.8
Ohio-1	32	28	87.5
Ohio-2	32	28	87.5
Ohio-3	32	29	90.6
Subtotal	96	85	88.5
TOTAL	251	187	74.5

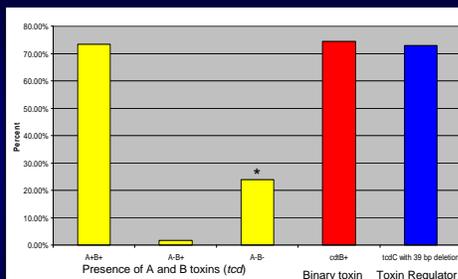
Nursery: 1/213 (0.47%); Slaughter: 0/94 (0%)

Antimicrobial Resistance



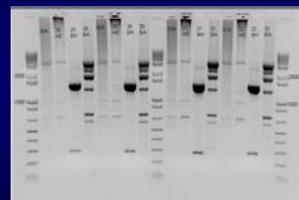
Breakpoints: Tet (>4), Met (>16), Ery (>2), Amp (>4), Van (>4), Cip (>32)

PCR for toxin genes



* 95% (39/41) of A-B- isolates are from the same farm, OSU #1

Toxinotyping Results



- 78% (49/63) of samples were **Toxinotype V**
 - One of the two hypervirulent strains of public health significance

In Summary,

- *C. difficile* is a public health important pathogen
 - Common in humans and various animal species
- New hypervirulent strains are emerging:
 - Excessive production of cytotoxin/ enterotoxins
 - Are multi-drug resistant
- **Piglets are commonly infected with one of the public health significant hypervirulent strains.**
 - O78, Toxinotype V
- Food safety and occupational significance need to be further investigated.
 - New project: Lejeune J. (PI- OARDC)
 - Multi-state project proposal- underway

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More info on project: <http://vet.osu.edu/idme1>