Mini-review

Clostridium difficile: An important pathogen of food animals

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Abstract

Human Clostridium difficile-associated disease (CDAD) is of unquestioned importance in humans, and has been a not-uncommon cause of enteric disease in horses, dogs, and ratites. Over the past 5 years, C. difficile has emerged as a major cause of neonatal enteritis in pigs. Piglets 1–7 days of age are affected, with gross lesions frequently including mesocolonic edema. Colonic contents may be pasty-to-watery and yellow, although some piglets are constipated or obstipated. Focal suppuration and segmental necrosis are seen on microscopic examination of cecal and colonic lamina propria, and exudation of neutrophils and fibrin into the lumen gives rise to the so-called volcano lesions. Results of one study revealed that more than one-third of piglets with enteritis were affected by C. difficile alone, while an additional quarter of affected piglets may have had mixed infections. C. difficile may be the most important uncontrolled cause of neonatal diarrhea in pigs.

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1. Introduction

Human Clostridium difficile-associated disease (CDAD) is responsible for \( \approx 25\% \) of all antibiotic-associated diarrheas [1], and the importance of CDAD in humans has prompted detailed study of the organism, its natural history, and its mechanisms of pathogenesis [2–5]. Antimicrobials disrupt colonic flora and ingested spores germinate in the colon [6,7]. Vegetative cells fill empty niches and produce toxins, and subsequent disease may present as diarrhea, colitis, or pseudomembranous colitis (PMC), often with systemic symptoms (fever, nausea, anorexia, and malaise) [6]. Fatal fulminant colitis, with ileus, toxic megacolon, and perforation, develops in a minority of patients [7,8], although the rate may be higher in patients infected with the recently emerged “epidemic” strains.

2. CDAD in pigs

Infection of pigs with C. difficile was first described more than two decades ago, a consequence of accidental exposure of gnotobiotic pigs [9]. Pseudomembranous colitis and large numbers of C. difficile were subsequently described in conventional pigs with naturally occurring CDAD [10]. Nearly 15 years later, C. difficile and its toxins were detected in an outbreak of CDAD in pigs \( \approx 5 \) days of age; affected piglets were dyspneic, and had mildly distended abdomens, scrotal edema, diarrhea, ascites (\( > 50 \text{ mL} \)), edema of the ascending mesocolon, and hydrothorax [11]. Microscopically, the distinctive lesions were severe ascending colonic submucosal and mesocolonic edema, with multifocal exudation of mucus, fibrin, and PMN aggregates. Outbreak-associated mortality as high as 16\% has been reported, mainly as a consequence of respiratory distress [12].

Our findings are similar [13–17]. Based upon examination of \( \sim 2000 \) individual diseased piglets, the case definition for porcine CDAD includes piglets 1–7 days of age with a history of scouring since shortly after birth. Litters from gilts and sows are affected. Gross lesions usually include moderate-to-severe edema of the mesocolon, and colonic serosal edema is common; pseudomembrane formation is uncommon, and the yellow plaques seen in human disease are not observed in affected piglets. Colonic contents are frequently pasty-to-watery and
yellow, although some piglets are constipated or obstruc-
ted. In an infected farrowing facility, toxins can be
detected in about two-thirds of litters and one-third of
individual pigs (92). Piglets without enteric signs may also
be toxin positive [13,18,19]. Scattered foci of suppuration
in cecal and colonic lamina propria are seen upon
microscopic examination, with segmental erosion of the
mucosa; neutrophil accumulation in the mesocolon is
common. Exudation of neutrophils and fibrin into
the lumen gives rise to the so-called volcano lesions
(Fig. 1). There are usually no remarkable lesions in small
intestine [17].

Since 2000, CDAD has been documented as a major
cause of enteritis in neonatal pigs [14–17,20–22]. There are
no accurate estimates of incidence and prevalence industry-
wide, but CDAD is the most commonly diagnosed cause
of neonatal enteritis throughout swine-producing areas of the
US. Examination of ~1000 live piglets with enteritis has
revealed toxins A (TcdA) and B (TcdB), without involve-
ment of other agents of enteritis, in 34.1%, and an
additional 24.3% of piglets were toxin positive and
also yielded some other agent (e.g., C. perfringens type A,
E. coli, or rotavirus) associated with porcine neonatal
enteritis. A random sample of 10 herds (32 litters per herd,
pooled sample of 5 pigs per litter) from a 13 herd
commercial farrow-to-wean system revealed 47.6% toxin
positivity across herds, with a range of 0–97%. More than
half of these herds were thought by producers and
veterinarians to be non-affected, even where piglets were
diarrheic. Combined with the findings of diagnosticians
across the US, these results suggest that C. difficile is
perhaps the most important uncontrolled cause of neonatal
diarrhea in pigs.

Porcine CDAD has been reproduced by administration
of pure cultures of C. difficile [13,21]. Pigs inoculated orally
with vegetative cells or spores develop characteristic signs
of CDAD, with mild-to-moderate mesocolonic edema and
pasty-to-watery, yellow colonic contents. All had mild
neutrophilic typhlocolitis with scattered apoptosis of
individual enterocytes on the mucosal surface, and were
culture positive for C. difficile and toxin positive by EIA.
Isolates from affected piglets were PCR-positive for both
toxin genes and were of the same ribotype as that of the
inoculating isolate [13].

3. Pathogenesis of CDAD

Clinically relevant strains of C. difficile usually produce
toxins A (TcdA, 308 kDa) and B (TcdB, 270 kDa),
members of the family of large clostridial cytotoxins
[23–25]. The former is enterotoxic and the latter is a potent
cytotoxin. They share an amino-terminal enzymatic
domain, a hydrophobic region believed to be involved in
translocation through endocytic vesicles into the cytosol,
and a carboxy-terminal domain containing the so-called
clostridial repetitive oligopeptides (CROPs) [26,27]; the
latter mediate toxin binding to target cells [23–27].
TcdA+/TcdB− strains [28–31] are recovered from piglets,
but results of toxin-binding studies have revealed that
TcdB fails to bind to tissues of neonatal pigs [13]. tcdA and
tcdB are located about 1 kb apart on a 19 kbp pathogeni-
city island [32].

TcdA and TcdB inactivate, by monoglucosylation, Rho
subfamily proteins, low MW GTP-binding molecules
involved in regulation of the F-actin cytoskeleton [25].
Disaggregation of polymerized actin leads to opening of
tight junctions and cell death. In humans, TcdA/B cause
release of proinflammatory mediators and cytokines,
leading to PMN chemotaxis and fluid secretion [33].
Patchy epithelial necrosis is common, and this can become
diffuse, with ulceration and pseudomembrane formation
[34]. Lesions are usually much more mild in piglets, and
gross mucosal lesions are usually absent.

As noted, C. difficile and its toxins can be found in non-
diarrheic piglets [13,35,36]. In one such group, 74% were
TcdA/B positive but had normal feces or were constipated
[19,36,37]. It is common to find typical lesions in these non-
diarrheic piglets. Some piglets present with systemic signs
[11], including mild depression, lack of appetite, cachexia,
and anorexia, without diarrhea. Most recover quickly, but
lack of nursing often leads to agalactia in sows. The only
diagnostic findings are toxins in cecum and colon and
typhlocolitis consistent with CDAD [13,19,35]. Micro-
scopic lesions in the colon are more common in piglets with
high levels of toxin [13,18,19]. Weaning weights in
surviving piglets are ≈1–2 lb per pig less than average [35].

4. Diagnosis

Bacteriologic culture often yields heavy growth of
C. difficile from affected tissues. However, as with human
CDAD, diagnosis of porcine CDAD is based primarily

Fig. 1. Suppurative focus in colonic mucosa of a piglet with CDAD.
Release of neutrophils into the lumen forms the so-called “volcano
lesion”.
upon detection of TcdA/B in stools or intestinal contents [6,7,38]. Comparison of cytotoxicity neutralization to a commercial enzyme immunoassay (EIA) revealed an overall correlation of 88% [20]. Sensitivity and specificity were 91% and 86% (comparable to experience in diagnosis of human CDAD) [38,39], leading to a conclusion that EIA is a suitable aid to diagnosis of porcine CDAD [40,41]. Toxin detection in fecal samples obtained by rectal swab is useful for ante-mortem diagnosis, in that results are 92.8% correlated with those from examination of intestinal contents [13]. There are other causes of mesocolonic edema, so the positive predictive value of mesocolonic edema for CDAD is only about 50%.

5. Prophylaxis and therapy

We tested the antimicrobial susceptibility of 80 porcine strains of C. difficile, using NCCLS methodology in an agar dilution format [42] (Table 1). The results suggest that tiamulin, virginiamycin, and tylosin in sow feed may be useful in limiting CD colonization and shedding, and that tylosin administered parenterally to piglets may be useful for prophylaxis or therapy. The bimodal distribution of susceptibility to tylosin suggests the existence of a resistance element.

The immune response to C. difficile infection in pigs has not been studied. However, antibodies against TcdA prevent binding, eliminate secretion and inflammation, and prevent clinical disease in mice and hamsters [33,43–52]; antiTcdB antibodies also have a role in protection against CDAD [10,53]. The concentration of antitoxic antibody in serum is inversely proportional to disease severity and relapse risk [29,54–57], and active or passive immunization against TcdA prevents clinical disease [43,44,47,58,59]. Thus, it may be that if a toxin-neutralizing response can be generated in sows, piglets can be passively protected by antibodies obtained from colostrum.

6. Conclusion

There are still many unanswered questions about CDAD in pigs. The role of toxins in pathogenesis is a prime area for inquiry, and such studies should yield information on appropriate methods and routes for immunoprophylaxis. The piglet may also provide an appropriate model for aspects of human CDAD.

Table 1

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC₅₀ (μg/mL)</th>
<th>MIC₉₀ (μg/mL)</th>
</tr>
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<tbody>
<tr>
<td>Bacitracin</td>
<td>&gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Cefitiofur</td>
<td>256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Tetracycline</td>
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<td>32</td>
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<tr>
<td>Erythromycin</td>
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<tr>
<td>Tiamulin</td>
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<td>8</td>
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<tr>
<td>Trimcin</td>
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<td>Tylosin</td>
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</tr>
<tr>
<td>Virginiamycin</td>
<td>0.25</td>
<td>2</td>
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</tbody>
</table>

References

[22] Post KW, Glenn Songer J, Jost BH, Glock RD, Holcamp A. The emergence of Clostridium difficile as a cause of porcine neonatal infection in pigs has...


