Clostridial Enteropathy in Lactating Outbred Swiss-derived (ICR) Mice

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Reports of severe enteric disease of unknown etiology affecting lactating mice have appeared in the literature. Clostridial disease similar to that seen in cattle and sheep on high-carbohydrate rations and caused by Clostridium perfringens has been suspected in these mouse outbreaks but has not been isolated from affected mice. The present report describes a severe, necrotizing enterocolitis associated with overgrowth of C. perfringens type A in lactating Swiss-derived (ND4) mice. Mice nursing large litters of pups in the second week of life were the most severely affected. The organism isolated from dead or moribund mice was positive by polymerase chain reaction assay for the gene for the C. perfringens α-toxin, but actual toxin production was not determined. The disease in this mouse colony was ameliorated by increasing the fat and calorie content of the diet of lactating dams, which each received 1 g peanut butter every 48 h.

A disease of unknown etiology associated with bloating, failure of lactation, and unexpected death in mice has been reported sporadically in multiple countries.11,16 The most complete description of the syndrome described an outbreak involving 50 mice of various strains that died or were euthanized over a period of 2 y.11 An outbreak of similar disease was described in 30 female SAM-P/6 mice.16 The cause of the deficient lactation syndrome was not identified in either report, but enterotoxemia due to clostridial infection or overgrowth was suspected in both instances.11,16 Previous reports have focused on the lesions in the most severely affected animals; however, the present report shows that clostridial overgrowth in mice can be severe or subclinical and can interfere with productivity and experimental variables. This report is unique because of the organisms that were identified, the link with lactational insufficiency, and treatment of the population with nutritional supplementation.

Clostridial organisms, especially the various serotypes of Clostridium perfringens, have long been recognized as a cause of enteritis and enterotoxemia in a variety of species. C. perfringens type A enterotoxemia is an important cause of food-born illness in people.15 Enterotoxemia caused by C. perfringens types B, C, and D is a common cause of morbidity and mortality in piglets, calves, and lambs. C. perfringens type D is associated with a syndrome in ruminants, particularly sheep, known as ‘overeating disease’ or ‘pulp kidney disease.’ The disease is most frequently identified in sheep fed high-calorie, high-carbohydrate feedlot rations or who are nursing older twin lambs.1,14

There have been few published reports of clostridial enteric disease in mice.3,13,17 One early report described enterotoxemia associated with C. perfringens type D in caesarean-derived, barrier-sustained suckling mice.3 A report of necrotizing enteritis in young RFM/M mice characterized the C. perfringens organism as simply ‘non-type-A’ because of lack of comparative antisera.15 C. perfringens types B and D were isolated from the gastrointestinal tracts of adult, germ-free BALB/c mice with enterotoxemia. Some of these mice also had left atrial enlargement with thrombus formation, and C. perfringens type B was isolated from the left atria.17 Here we describe an epizootic of necrohemorrhagic enteritis, lactational insufficiency, and bloating associated with clostridial overgrowth in a population of outbred mice. Included in this report are the clinical signs in the affected mice, the effect of the disease on the behavior of the affected dams and their pups, necropsy findings, and results of microbiologic investigation.

Methods and Materials

Animals and husbandry. The Institutional Animal Care and Use Committee of the University of Wisconsin–Madison approved all experimental procedures. The founders were male (n = 112) and female (n = 112) Hsd:ICR mice (Harlan Sprague Dawley, Indianapolis, IN). The mice were housed in a conventional facility in standard, open-topped polycarbonate cages on woodchip bedding; were fed Rodent Diet (W) 8604 (Harlan Teklad, Madison, WI) and water ad libitum; and were maintained in a room on a 12:12-h photoperiod. The original colony was expanded through 2 generations of random, nonsibling pairing. The resulting progeny were assigned randomly to 1 of 8 closed populations: 4 Control lines and 4 lines that underwent selection for high voluntary wheel-running (High-Runner lines). In each generation, male and female offspring from 10 different litters per line were tested at about 6 to 8 wk of age for voluntary wheel running. Breeders for each of the High-Runner lines were chosen from the mice with the greatest (within-family) average number of revolutions on days 5 and 6 of a 6-d wheel-running trial. Breeders for each of the Control lines were chosen randomly within family. Sibling mating was disallowed in all lines.19

Routine serologic testing of mice from this colony indicated that they were negative for the following murine pathogens: Mycoplasma pulmonis, Sendai virus, mouse hepatitis virus, pneumonia virus of mice, reovirus type 3, Thieler virus, ectromelia, mouse adeno-virus, polyoma virus, lympholytic choriomeningitis virus, cytomegalovirus, murine rotavirus, and CAR bacillus. Sporadic seropositive reactions against murine parvoviruses have been documented in this colony.

Physical, behavioral, and clinical assessment. Bloating and unexpected death in lactating mice had been observed for more than 1 y prior to the outbreak described in this report. In light of preliminary observations that the colony had experienced a cumulative 24% death loss in lactating dams in the 10 genera-
CONDITION OF DAM

<table>
<thead>
<tr>
<th>Maternal ID #</th>
<th>Date</th>
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Maternal Body Condition Score
1. Full, rounded flanks when viewed from behind, well above backbone
2. Flanks rounded, but about even with backbone
3. Flanks slightly concave, top of rump below backbone
4. Flanks severely concave

Maternal circumference measured from widest point ______

Degree of discoloration of intestines as seen through the skin
1. Normal, cannot see intestinal discoloration
2. Mild, minimal discoloration
3. Moderate, intestines red to dark
4. Severe, intestines are dark to black and can be easily visualized through the skin.

Tail Veins
1. Normal, pink to slightly red tail vessels
2. Minimal discoloration and some engorgement
3. Moderate, veins are red and significantly engorged
4. Severe, veins are black and significantly engorged

Condition of hair coat
1. Smooth, unruffled, well-groomed hair coat
2. Haircoat is ruffled, sticky or unkempt-looking

Fecal staining or loose feces in cage
1. No
2. Yes

Activity
1. Normal, moves easily, eating, grooming, shows interest in pups
2. Slightly depressed, but still active and alert and eats
3. Reluctant to move, still interested in pups, may eat
4. Reluctant to move, not very interested in pups, not eating

Body mass ______ g

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Figure 1. Physical assessment form for lactating female mice.

Prior to the outbreak described here, the investigators and veterinary staff conducted a detailed study of reproduction in females of the 27th generation of this closed colony. We studied 84 of the dams with first litters and reevaluated 65 of the surviving dams through a second litter several weeks later. Data collected from the dams included body condition score (on a scale of 1 to 4), abdominal circumference, presence and degree of darkening of abdominal viscera and tail veins, condition of hair coat, presence of fecal staining of the perineum or diarrhea in the cage, visual assessment of homecage activity, and body mass (Figure 1). Data collected from the litter included the age of the pups at the time of examination, number of pups in the litter, number of pups born alive, number of pups surviving to the second litter, and number of pups remaining at the time of examination.
## CONDITION OF PUPS

### I. Pup Body Condition Score
1. Full, rounded flanks when viewed from behind, well above backbone
2. Flanks rounded, but about even with backbone
3. Flanks slightly concave, top of rump below backbone
4. Flanks severely concave

Condition of hair coat
1. Smooth, unruffled, well-groomed hair coat
2. Hair coat is ruffled, sticky or unkempt-looking

Activity
1. Normal, active if jostled, but otherwise stay in the pile, act satiated
2. Active if jostled, still in the pile, but want to nurse avidly
3. Less active if jostled, but stray from the pile to try to nurse
4. Barely moving, shows little interest, even in nursing the dam

Age of pups ____ days
Total mass of pups ____ g
Number of pups in litter ____
Number of dead pups ____

### II. Pup Body Condition Score
1. Full, rounded flanks when viewed from behind, well above backbone
2. Flanks rounded, but about even with backbone
3. Flanks slightly concave, top of rump below backbone
4. Flanks severely concave

Condition of hair coat
1. Smooth, unruffled, well-groomed hair coat
2. Hair coat is ruffled, sticky or unkempt-looking

Activity
1. Normal, active if jostled, but otherwise stay in the pile, act satiated
2. Active if jostled, still in the pile, but want to nurse avidly
3. Less active if jostled, but stray from the pile to try to nurse
4. Barely moving, shows little interest, even in nursing the dam

### III. Pup Body Condition Score
1. Full, rounded flanks when viewed from behind, well above backbone
2. Flanks rounded, but about even with backbone
3. Flanks slightly concave, top of rump below backbone
4. Flanks severely concave

Condition of hair coat
1. Smooth, unruffled, well-groomed hair coat
2. Hair coat is ruffled, sticky or unkempt-looking

Activity
1. Normal, active if jostled, but otherwise stay in the pile, act satiated
2. Active if jostled, still in the pile, but want to nurse avidly
3. Less active if jostled, but stray from the pile to try to nurse
4. Barely moving, shows little interest, even in nursing the dam

Figure 2. Physical assessment form for pups.
litter and the total mass of the pups in grams, and presence and number of dead pups. Three pups were chosen at random from
the litter and were assessed for body condition score, condition
of their hair coat, and activity (Figure 2). Pups were chosen
by placing the entire litter in an opaque container to obtain a
composite weight. Then an assistant removed 3 pups from the
container without observing the pups.

Breeding in this colony was temporally regulated, so pups
were born, weaned, tested, and euthanized at discrete inter-
vals. In order to accomplish this objective, breeding pairs were
formed on a single day, females were observed for vaginal plug
formation and the males were removed until the litter was
weaned. This breeding scheme allowed researchers to assess
these physical outcome measures of the dams and pups at the
time of the epizootic and to resample many of the surviving
dams with the next litter of similarly aged pups after the insti-
tution of a nutritional supplement. In light of the preliminary
clinical and pathologic evaluations of the dams and pups dur-
ing the epizootic, the decision was made to provide the female
breeders with a high-calorie nutritional supplement consisting
of approximately 1 g peanut butter (Jif Creamy Peanut Butter,
JM Smucker, Orrville, OH) every 48 h from parturition through
weaning at 21 d because the initial necropsies indicated that
affected dams were in very poor body condition. Peanut butter
was chosen as the supplement because it is high in fat, inexpen-
sive–readily available, and palatable for mice.

The frequency of maternal care was assessed using a scan-
sampling technique12 during the light (1–3 h after lights on) and
dark (1–3 h after lights off) periods when the litters were 9 and
16 d old. Dams were randomly assigned an observation order
at the beginning of each observation period. A single observer
watched each dam for 10 s as timed by a flashing LED device,
and the dam’s instantaneous behavior at the 10-s mark was re-
corded. Each dam was scanned 25 times during an observation
period with 5 min between scans on the same animal. A more
complete description of maternal behavior in these animals is
included in Girard and colleagues.3

Statistical analysis. Statistical analyses were performed using
SAS (SAS Institute, Cary, NC) and significance threshold was
set at $\alpha = 0.05$. The relationship of dam condition to various
behavior and outcome measures was examined by Pearson
product-moment correlation. Paired t test was applied to test
for changes between first and second litters.

Results

Characteristics of the clinical disease. Adult lactating female
mice were affected preferentially. No male breeder mice died
or became ill, despite being housed in open-top caging in the
same room as the affected females. Frequently female mice
with litters that were observed in the morning to have been
active and alert were found dead (with variable numbers of
dead pups) at the afternoon check. Ten female mice were found
either dead or moribund out of a total at-risk population of 84
mice, giving a prevalence of severe disease of 11.9%. Of these,
8 were found dead, 1 was euthanized, and 1 recovered after
penicillin treatment. Upon gross visual inspection, the carcasses
had black skin, especially over the abdominal area. Pups were
alive but cold and hungry. The average litter age at the onset
of the disease was 14.25 d, and the average litter size of the
severely affected animals was 10.75 pups. Some live mice were
observed to have dark viscera visible through the abdominal
wall. These mice often were bloated and had darkening of the
feet and tail vessels.

Physical outcome measures. Dam condition scores ranged
from 7 (poorest condition) to 18 (best condition). The mean com-
posite dam condition score for dams with their first litter was 10,
indicating poor condition (Table 1). Dam score was positively
 correlated with litter size (number of pups; $n = 84$, Pearson $r = 0.24$, $P < 0.0001$; Figure 3). Pup condition scores ranged from 12
(poorest) to 24 (best). The mean composite pup condition score
in the first litters was about 16, indicating poor condition. Pup
score was significantly and positively correlated with dam score:
pups in poorest condition were from the poorest condition dams
($n = 84$, Pearson $r = 0.28$, $P < 0.0001$; Figure 4).

Ten dams died before weaning the first litter. In 8 cases, the
orphaned pups survived to weaning with self-bottle-feeding
(KMR4 Kitten Milk Replacer, PetAg, Hampshire, IL; adminis-
tered in a 200-ml glass bottle with an elongated drinking tube
and refreshed twice daily). In 2 litters, all pups and the dam
were found dead. Surviving dams were paired again with their
original mates, and 65 females delivered a second litter. Mean
composite dam condition score was significantly (paired t test,
$P < 0.0001$) improved in these multiparous females during the
second litter ($8.45 \pm 1.42; n = 65$) from scores recorded in those
same mothers from the first litters ($10.35 \pm 2.78; n = 65$). Matern
al condition score at the second litter was not correlated with
score at first litter (Pearson $r = 0.032$, $P > 0.7$). Second litters were
significantly larger in number of pups (paired t test, t = 3.54, P < 0.001), but average pup mass did not differ between first to second litters (paired t test, t = 1.74, P > 0.08). Pup age at time of assessment did not differ between the 2 litters (paired t test, t = 1.23, P > 0.1).

Behavioral outcome measures. Behavioral observations of the dams and pups were performed during the epizootic and during the next breeding cycle after provision of a nutritional supplement to the lactating mice. Scores generated from the scan-sampling technique ranged from 0 (nursing never observed) to 25 (nursing observed at every interval) in samples at litter age of 9 and 16 d.5 Nursing scores were highest in the most severely affected dams. With nutritional supplementation, nursing scores for the second litters decreased in both the 9- and 16-d scan samples (paired t test, t > 4.58, P < 0.01). Although dams spent less time nursing second litters, second-litter pups were as large at weaning as first-litter pups (Table 1).

Pathology. A total of 9 mice were submitted for necropsy. Grossly, mice found dead or moribund were dehydrated and had sunken eyes. The carcasses were bloated and autolyzed, and black abdominal viscera were visible through the abdominal wall. The tail vessels were dark and dilated, and some animals had darkening of the feet. The mammas were prominent and frequently were red (indicative of inflammation) or had the same dark discoloration as the tail vessels and extremities. When a carcass was opened, the gastrointestinal tract often was distended with gas and filled with fetid red-brown to green contents throughout the small and often the large intestinal segments. There was little body fat on the carcasses (Figure 5).

Formalin-fixed tissues from several of the mice that were euthanized in a moribund state or were found dead were submitted for standard histologic analysis. The principal findings were severe necrohemorrhagic enteritis with a mixed inflammatory cell infiltrate (Figure 6 A). A tissue Gram stain of the cecum was done. Numerous gram-positive rods were present over the surface of the mucosa (Figure 6 B). Swabs for aerobic and anaerobic culture were obtained at gross necropsy from 4 of the dead or moribund mice. Aerobic cultures grew moderate to heavy numbers of expected flora. The anaerobic cultures from 4 individual mice from 2 separate submissions grew *C. perfringens* type A, generally in large numbers.

Polymerase chain reaction analysis for toxin-producing genes was done on 1 of the *C. perfringens* isolates on a fee-for-service basis in the laboratory of Glenn Songer (University of Arizona) according to published methods.4 The findings indicated that

The isolate was capable of producing the clostridial α toxin. Polymerase chain reaction analysis was negative for amplification of the β, ε, and α toxin genes. The clinical and pathologic data from this mouse breeding colony are consistent with an outbreak of necrohemorrhagic enteritis resulting in the death of 8 mice due to toxiosis. Other mice had evidence of less acute illness that could be attributed to clostridial overgrowth.

Long-term follow-up. Deaths from suspected or confirmed clostridial disease have not occurred in this mouse colony during the 3-y period after the addition of the high-fat food supplement. After 3 y, most of this mouse-breeding colony was moved to a different institution. A few of the mice were moved to a different facility within the same institution and have remained free of disease for 6 y.

Discussion

The clinical features of this epizootic in mice were consistent with a disease caused by enteric clostridial overgrowth. Clostridial enterotoxemia was suspected in previous reports of abdominal distention associated with unexpected death in lactating mice in light of clinical and postmortem findings, but the organism was not identified by anaerobic culture.11,15 Gram stains of intestinal contents were made in 1 clinical report to try to rule out clostridial disease, but this assay is not a very sensitive method of identification.11 The major risk factors for more severe disease in these mice were female gender and the presence of a large litter in its second week of life. Clostridial organisms have been suggested as the cause of unexpected death in lactating mice, but the etiology had never been confirmed by isolation of an organism.11,15 Clostridial organisms have previously been isolated from mice, both from neonates13 and adult germ-free mice with enteric disease.17

Large litters are a characteristic of Swiss mice and of laboratory mice derived from the original Swiss stock, including ICR mice. The record number of pups weaned from a single litter in a female Swiss mouse is 36.57 Large litters are an efficient use of animal resources. However, the metabolic demands of a large litter require the dam to increase her rate of energy consumption.6 At peak lactation (about 14 d postpartum), the rate of food consumption is 3 to 4 times higher than that during gestation,7 and the rate of gross energy intake is 7.5 times higher than during resting metabolism.10 One report of an outbreak of enteric disease in lactating female mice found primiparity was a risk factor for developing the disease.11

The authors of
that report reasoned that because the mice were rebred on the postpartum estrus, they were nursing their first litter and carrying their second while they were still physically immature. In contrast, although most of the affected mice in the outbreak we describe here were primiparous, they were not rebred on the postpartum estrus. The high metabolic demand engendered by large litters could have sufficed to produce the conditions necessary for overgrowth of toxigenic organisms without the additional risk factor of combined lactation and pregnancy. Other factors that could have contributed to this outbreak include environmental factors such as the degree of soil contamination of the food or bedding (because it was not autoclaved before use) and seasonal fluctuations in temperature or humidity that could have allowed the organism to grow to larger numbers in the environment, especially in the bedding. In addition, the lack of barrier facilities may have facilitated spread of the organism once it was established in the mouse colony.

The classic ‘overeating disease’ of ruminants has been associated with C. *perfringens* type D, and this organism also has been shown to cause disease in mice. For example, *C. perfringens* type D was isolated from an outbreak of enteric disease in suckling mice, and types B and D have been isolated from an outbreak in germ-free adult BALB/c mice. However, polymerase chain reaction analyses for toxin genes from the *C. perfringens* organisms that we isolated from the mice from our colony were positive for the type A toxin only. It seems possible that α toxin alone can cause disease in mice. The most definitive test for the toxigenicity of clostridial isolates is a mouse protection assay. This assay requires at least 50 ml of intestinal contents from the affected animal. It was not possible to obtain enough material from the mice, even when the gut contents of multiple animals were pooled. The signalment in these mice resembled that of one of the known risk groups for ruminant overeating disease due to *C. perfringens* type D but the pathologic
features and results from the polymerase chain reaction assay were consistent with *C. perfringens* type A. Definitive testing for toxigenicity of *C. perfringens* isolates from mice will have to await methods that can be applied to small amounts of material.

None of the previously reported investigations of clostridial disease or lactational insufficiency addressed the issue of subclinical disease. In the epizootic we report, morbidity in the dams and pups was quantified. In addition, we collected behavioral data, both during the epizootic as well as during the subsequent litter, after the dams received nutritional supplementation with a high-fat food (peanut butter). All physical outcome measures for both dams and pups improved between the first and second litters. Nursing scores also decreased from the first to the second litter, presumably because the pups did not need to nurse for as long to be satiated (Table 1). Treatment and prevention of overeating disease in ruminants generally is achieved by increasing the amount of fiber in the diet.\(^1\),\(^4\),\(^13\)\(^8\) This practice lowers the amount of energy per gram of diet and reduces the amount of carbohydrate entering the small intestine. A high-fat food supplement like peanut butter would increase the amount of energy per gram of diet but would be expected to have the same net effect of decreasing the amount of starch entering the small bowel.

Feeding a high-fat food supplement, as opposed to increasing the roughage content of the diet, was particularly advantageous in our case, because many of the mice had poor body condition scores when first examined (Figure 3). We conclude that the nutritional supplement was effective in preventing morbidity and mortality in the lactating mice. Supplementation with a high-fat food item was an easy, inexpensive method of preventing enteric clostridial disease in lactating mice. In one report, mice with enteric clostridial disease were treated successfully with tetracycline delivered in their water.\(^13\)\(^8\) We considered antibiotic treatment for the mice during the outbreak, but nutritional supplementation was chosen as therapy because the investigator believed that the supplement was less likely to interfere with ongoing research.

Alternative explanations for this finding of improved condition with supplementation include acquired immunity, physiologic priming, and selection for resistance. It is possible that the mice became immune to the clostridial organism and that this immunity prevented overgrowth during the lactation with the second litter. Dams can become physiologically primed during the first lactation and can increase their maximal rate of energy intake during lactation for the second litter as compared with maximal intake in primiparity.\(^1\) Both of these explanations are unlikely. Immunity to *C. perfringens*, both natural and vaccinal is incomplete and short-lived.\(^9\) In addition, this colony had experienced similar instances of high maternal mortality prior to the epizootic documented here. After dietary supplementation was instituted for all lactating dams, subsequent outbreaks of clostridial disease did not occur in the colony. Finally, improvement in the health of the dams and pups was observed using repeated-measures analyses of assessments performed on both first and second litters. The use of the repeated-measures design meant that the most severely affected mice—those that died before the birth of their second litter—were excluded from the analysis. The test was more conservative because of their exclusion.

Physical and behavioral measures of morbidity do not have the definitive nature of pathologic and microbiological data, but they do serve as an adjunct to these evaluations. Controlled studies of *C. perfringens* pathobiology would be important in determining the importance of this organism in lactating mice. Controlled studies would allow for investigations to be conducted that would be difficult to perform during an outbreak of clinical disease. Larger numbers of animals could be evaluated by pathologic and microbiologic means; gut or fecal samples could be obtained from subclinically affected mice, uninfected mice and severely affected individuals; and nutritional or antimicrobial interventions could be evaluated more effectively.

There have been few reports of spontaneous enteric disease in mice caused by clostridial organisms. This is the first report that has described microbiologic, pathologic, body condition and behavior data in a population of adult lactating mice with clostridial enteropathy. The primary risk factor for the disease was high metabolic demand in lactating mice. Such high demand may result from rebreeding young inbred mice on the postpartum estrus\(^11\) or from older litters of large numbers of pups in outbred mice, as we described here. High-carbohydrate diet and lack of a robust normal flora also may contribute to the development of the disease. The mice in this study were also evaluated for their response to a high fat nutritional supplement. Supplementation is inexpensive and in our study, an effective intervention.

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**References**