

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 sub-clinical 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-borne 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 'pulpy 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.¹³ *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 wood-chip 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, Theiler virus, ectromelia, mouse adenovirus, polyoma virus, lymphocytic 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-

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CONDITION OF DAM	
_____	Maternal ID # _____ Date _____
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	

Figure 1. Physical assessment form for lactating female mice.

tions 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),²¹ 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

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

Age of pups _____ days

Total mass of pups _____ g

Number of pups in litter _____

Condition of hair coat

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

Number of dead pups _____

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

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.

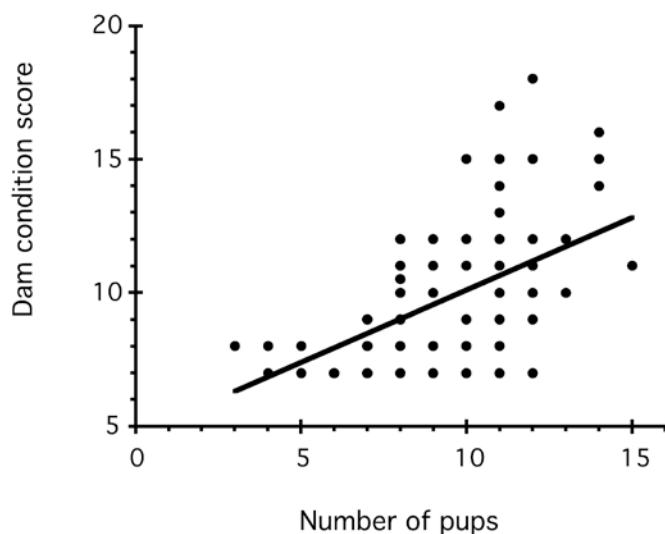


Figure 3. Composite dam condition score is positively correlated with litter size in first litters.

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 intervals. 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 institution of a nutritional supplement. In light of the preliminary clinical and pathologic evaluations of the dams and pups during 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, inexpensive-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 recorded. 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.⁵

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.

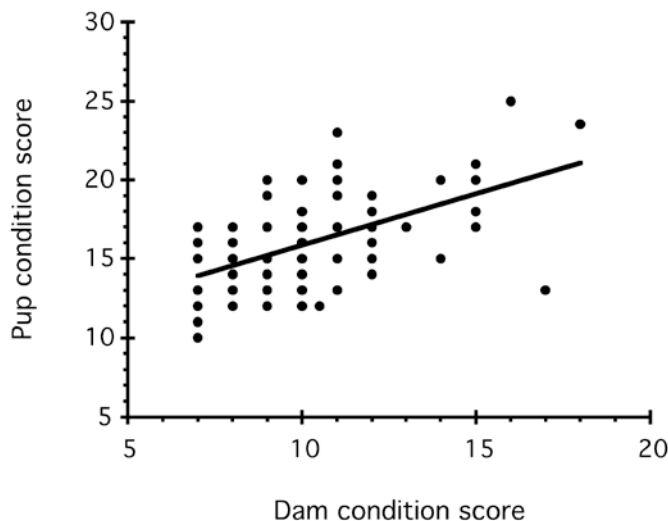


Figure 4. Composite pup condition score is positively correlated with the composite dam condition score in first litters.

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 composite 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; administered 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 ± 1.42 ; $n = 65$) from scores recorded in those same mothers from the first litters (10.35 ± 2.78 ; $n = 65$). Maternal condition score at the second litter was not correlated with score at first litter (Pearson $r = 0.032$, $P > 0.7$). Second litters were

Table 1. Dam and pup condition at assessment in first (n = 84 dams) and second litters (n = 65 dams)

	First litter		Second litter	
	Mean	1 standard deviation	Mean	1 standard deviation
Dam mass (g)	45.23	3.03	44.60	4.10
Dam age (d)	90.3	2.31	134.2	3.52
Dam condition score	10.09	2.63	8.45 ^a	1.42
Nursing score (9 d)	13.12	4.83	12.14 ^{a,b}	4.61
Nursing score (16 d)	17.23	4.23	14.95 ^{a,b}	4.79
Litter mass (g)	65.93	13.08	76.92 ^a	12.53
Litter age (d)	15.24	2.65	14.63	1.88
Litter size (n)	9.94	2.25	11.16 ^a	2.92
Pup mass (g)	6.82	1.31	7.21	1.43
Pup condition score	15.91	3.09	10.32 ^a	1.85

Dam condition ranged from 7 (best condition) to 18 (poorest condition). Nursing score, assessed at litter age of 9 and 16 d, ranged from 0 (nursing never observed) to 25 (nursing observed in every interval). Pup condition ranged from 10 (best condition) to 25 (poorest condition).

^a $P < 0.05$ (paired t test) for first versus second litters.

^bSample size was 24 dams for behavioral observations in second litters.

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 mammae 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 ι 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 toxicosis. 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 neonates^{3,13} 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.^{5,7} 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,² 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

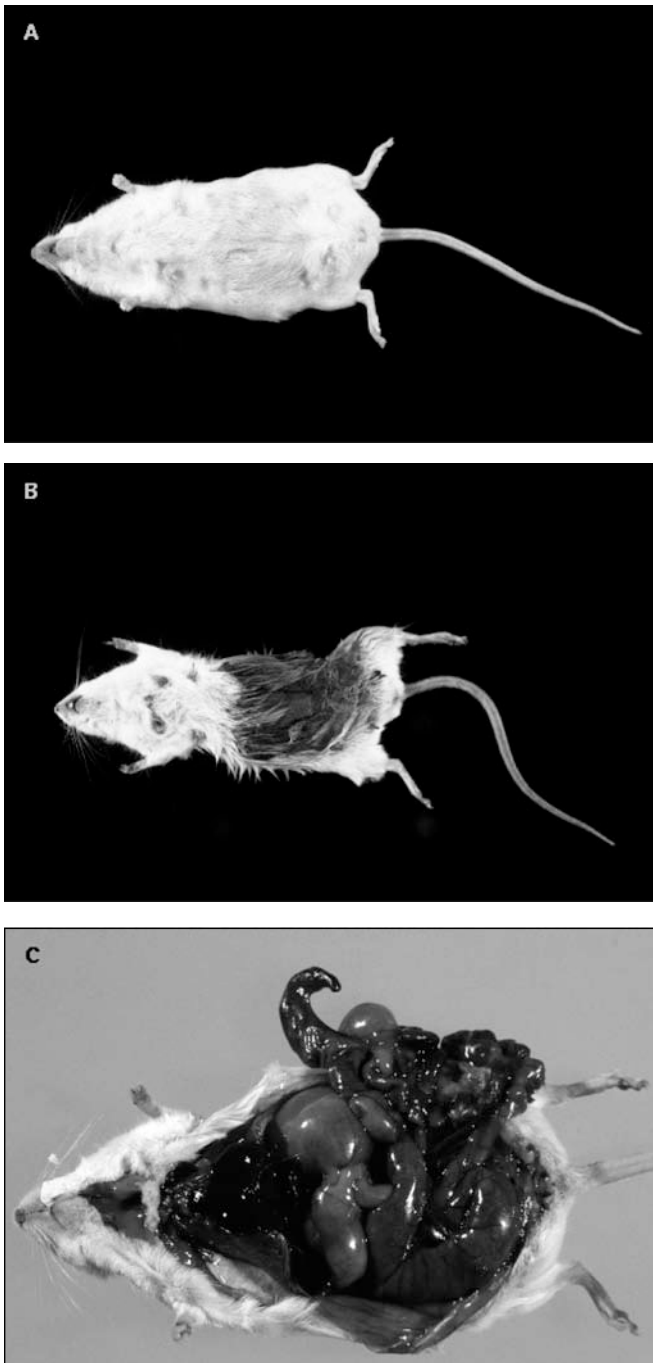


Figure 5. Photographs of severely affected mice. (A) Albino Swiss-derived mouse that died unexpectedly during late lactation. The tail artery is dark, and dark abdominal visceral can be seen through the abdominal wall. (B) Ventrum of affected mouse with dark, necrotic skin around the mammae and intestinal contents staining the abdomen. (C) Carcass with the abdomen open: the intestinal tract, especially the cecum, is dark and distended with gas and fluid. There is severe hyperemia of the liver and lungs.

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 ad-

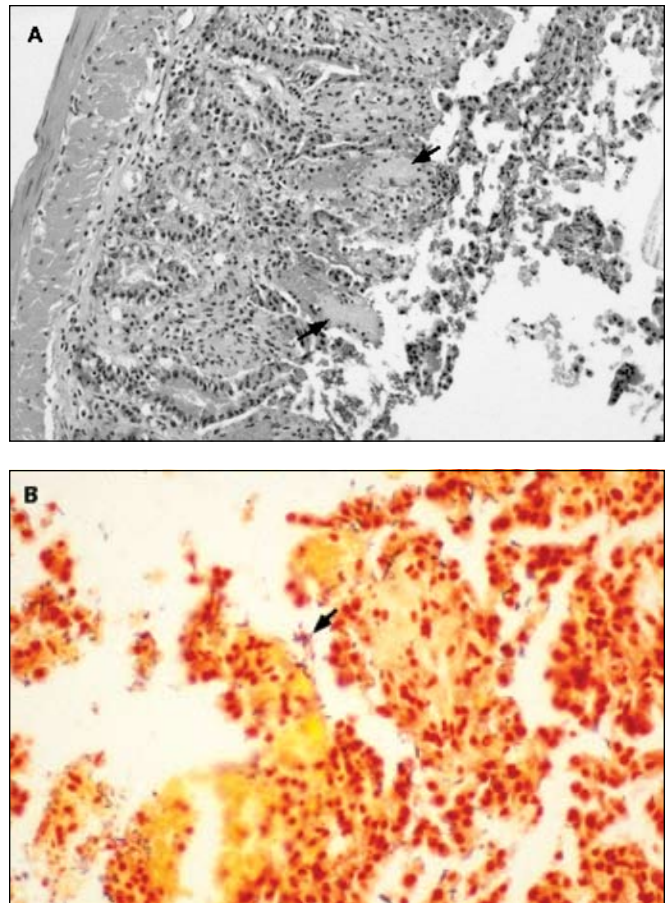


Figure 6. Histopathology of the cecum of a mouse with *C. perfringens* type A overgrowth. (A) Section of cecum showing hemorrhage and necrosis of villi (arrows). Hematoxylin and eosin stain; magnification, $\times 400$. (B) Gram-stained serial section of cecum from the same mouse with large numbers of long, gram-positive rods growing in close association with the intestinal villi (arrow). Magnification, $\times 1000$.

ditional 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,^{1,14} 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,³ and types B and D have been isolated from an outbreak in germ-free adult BALB/c mice.¹⁷ 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.¹⁴ 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,^{14,18} 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 sub-clinical 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.^{1,14,18} 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 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.⁹ Both of these explanations are unlikely. Immunity to *C. perfringens*, both natural and vaccinal is incomplete and short-lived.²⁰ 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 estrus¹¹ 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.

Acknowledgments

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