Brief Communication Communication brève

Clostridial abomasal disease in Connecticut dairy calves

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Abstract — Over 2 years, 24 dairy calves died of emphysematous abomasitis and abomasal bloat. Anaerobic cultures of necrotic abomasal mucosa yielded *Clostridium perfringens* from 10 of 15 calves. *Sarcina* were observed in 17 of 22 examined histologically. A change in the antibiotic regimen for newborns and improved sanitizing of feeding utensils eliminated further losses.

Résumé — Maladie abomasale à *Clostridium* chez les veaux de troupeaux laitiers du Connecticut. Pendant 2 ans, 24 veaux de troupeaux laitiers sont morts d'abomasite emphysémateuse et de ballonnement de l'abomasum. Les cultures anaérobies des muqueuses abomasales nécrotiques ont révélé *Clostridium perfringens* chez 10 des 15 veaux. *Sarcina* a été observé par examen histologique chez 17 des 22 animaux. Un changement du régime d'antibiotiques pour les nouveaux-nés et une désinfection améliorée des ustensiles d'alimentation ont prévenu de nouvelles pertes.

(Traduit par Isabelle Vallières)

Can Vet J 2009;50:857–860

In December 2001, a large central Connecticut dairy farm began to recognize unusual acute deaths in calves. Losses occurred sporadically, causing us to necropsy 24 calves over 2 y. Our findings of extraordinary abomasal disease are reported herein.

The farm that experienced these losses was comprised of 2 milking herds, each of 530 head, and a calf-rearing operation that grew 500 young stock for herd replacements each year. At each of the 2 herds, calves were born in a calving stall with clean wood shavings as bedding. Calves were not allowed to nurse their dams; they were fed pooled colostrum and then transported by a small truck to the calf-rearing operation. In the past, calf losses had been 1% to 2 % per year, and they were from a variety of causes, such as calfhood pneumonia or colibacillosis.

The calf barn was 90-m long and contained 86 individual 1.2 m × 2.4 m calf pens. Colostrum from the 2 herds was pooled, held refrigerated in 1-gallon glass jars with metal lids, and fed to newborn calves for 72 h [3 to 4 quarts (2.8 to 3.8 L) soon after birth, and then 2 quarts per feeding, twice daily], after which they were switched to a commercial dried whey-based milk replacer of 20% protein, 20% fat content. The latter was fed twice daily from clean plastic buckets at a level of 10% body weight per feeding. A probiotic preparation with vitamins (Convert Probiotic; Lallemand Animal Nutrition, Milwaukee, Wisconsin, USA) was mixed with the powdered milk replacer, 1 lb (0.4 kg) per 100 calves per day. Water was provided ad libitum after each feeding. Aqueous procaine penicillin G (Aquacillin; IVX Animal Health, Fort Dodge, Iowa, USA), 900 000 units, was given PO for the first 3 d of life and then 1 500 000 units intramuscularly for 3 d more.

The farm had a rigorous immunization program, calves receiving a K99 + *Escherichia coli* and coronavirus antibody, PO, on the day of birth and bovine antiserum against *Arcanobacterium pyogenes, E. coli, Salmonella typhimurium, Mannheimia haemolytica* and *Pasteurella multocida* parenterally on the day of birth and at 1 week of age. Modified live IBR and PI 3 vaccine was given at 2 to 4 weeks of age.

In December of 2001, the first of what was to be a 2-year string of sporadic calf deaths occurred. Necropsy was done on a 14-day-old calf that died overnight, after having been normal at the evening feeding the day before. In this 45-kg calf, the rumen, reticulum, and abomasum were irregularly blackened with congestion and hemorrhage, the abomasum was greatly distended with thin brown fluid, and the abomasal folds were thickened with edema, contained gas bubbles, and were reddened or blackened with hemorrhage and necrosis, particularly over the greater curvature. Sixty percent of the abomasal wall was thickened and black, and the small intestine was segmentally reddened, sometimes fluid-filled, sometimes distended with gas.

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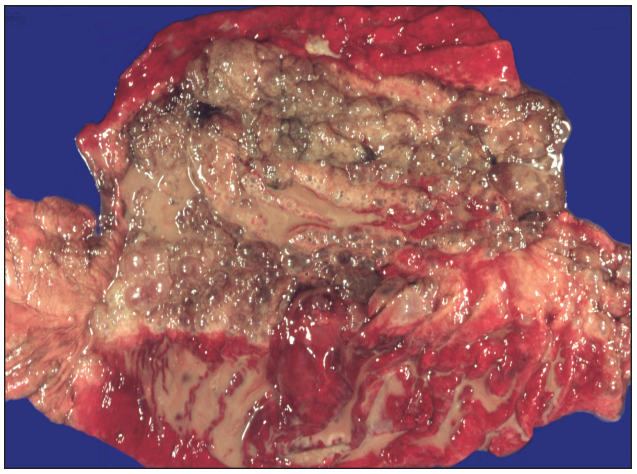

Figure 1. Abomasum from a 7-day-old calf, opened along the greater curve; pyloric antrum to the left. The mucosa is severely reddened and folds are thickened. (upper and lower portions); mucosa at omasal-abomasal orifice (to the right) and along the lesser curve is tan to brown and emphysematous.

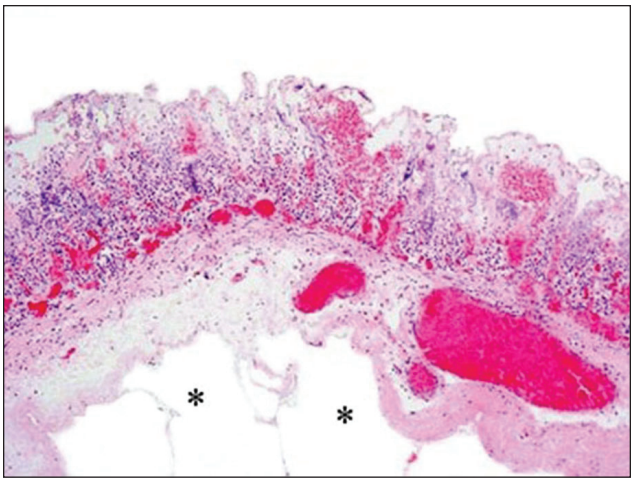

Figure 2. Abomasum from a 7-day-old calf. There is severe congestion and necrosis of the mucosa, and congestion and emphysema (asterisks) of the submucosa. Hematoxylin and eosin. Bar = 100 μm.

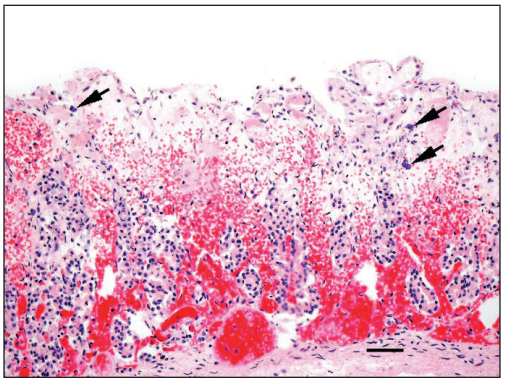

Figure 3. Higher magnification of a section of Figure 2. Abomasal mucosa shows severe congestion and interstitial hemorrhage, disintegration of gastric glands, and necrosis and edema at the surface. Arrows indicate clusters of *Sarcina.* Hematoxylin and eosin. Bar = 50 μm.

A few intestinal segments were focally blotched with ecchymoses. Because of previous concerns about salmonellosis at the farm, abomasal contents, small bowel contents, liver, spleen, and bile were cultured, all of which were negative.

A total of 24 calves died over 26 mo; all with variable abdominal distention and acute death (12 to 18 h of illness). The calves ranged in age from 4 to 42 d, and had abomasal bloat, emphysematous abomasitis (Figure 1), abomasal rupture, or emphysematous gastritis that included the abomasum and some part of the rumen or reticulum (Table 1). Some had segmental necrotic enteritis as well. Histologically, affected abomasums, and parts of the forestomachs had coagulation necrosis of the mucosal surface and emphysema of the mucosa and submucosa (Figures 2 and 3). There was often edema of the lamina propria and submucosa. Seventeen of 22 calves from which sections were taken for histology had organisms consistent with *Sarcina* sp. in the necrotic material that adhered to the damaged mucosa. Aerobic cultures of the necrotic abomasum or its contents revealed no pathogens; however, once anaerobic cultures (on blood agar, in 9% to 13% CO_2, < 1% O_2, at 37°C) were initiated *Clostridium perfringens* was recovered from 10 of the 15 tested (9 were not tested). Five isolates that were referred for typing (PCR for α, β, ε, ι, cpe, and β2 toxin genes) were uniformly type A (Arizona Veterinary Diagnostic Laboratory, Tucson, Arizona, USA).

On January 27, 2004 a farm visit was made to meet with the farm manager and calf-rearing manager to take a detailed history (described previously) and to test several feed items for clostridia. The samples were plated onto blood agar and incubated anaerobically at 37°C. One sample of probiotic and 2 samples of milk replacer were negative; however, 1 of 3 samples of pooled colostrum yielded *C. perfringens.* At a subsequent visit February 14, 2004, 17 environmental swab samples were collected and cultured anaerobically. Two surfaces, a back wall area, and a side partition of a calving pen, the bucket milker for taking colostrum from fresh cows, and the walls of the refrigerator holding jars of pooled colostrum yielded *C. perfringens,* type A. This latter organism, and one of the 5 from the calves, were positive for the β2 toxin gene.

At the time of the farm visits, it became clear that the antibiotics given to the newborn calves had been selected to control colibacillosis and were based on antibiograms of the past. Antibiograms then conducted on the type A *C. perfringens* under anaerobic conditions (Kirby-Bauer antibiotic sensitivity testing method, on Mueller-Hinton agar plates with blood) suggested that the antibiotics being given at the time favored the growth of *C. perfringens.* Antibiograms done on the last 7 *C. perfringens,* recovered from abomasal fluid or necrotic abomasal tissue, showed 3 of the 7 to be resistant to penicillin. A change was made, based on the antibiotic sensitivity; penicillin was replaced with ampicillin and oxytetracycline during the 1st week of life. Since the change was initiated, along with improved cleaning of milking utensils and colostrum jars, there have been no further losses.

The features in these calves resemble those reproduced by intraruminal inoculation of type A *C. perfringens* into calves, the latter, however, lacking abomasal emphysema (1,2). We

Table 1. Case data and microbiologic findings in 24 calves that died

Calf	Age (d)	Diagnosis	*Clostridia*	*Sarcina**
1	14	Emphysematous abomasitis	ND	+
2	7	Emphysematous abomasitis with perforation	ND	+
3	14	Abomasal rupture	ND	ND
4	10	Abomasal rupture	ND	ND
5	28	Rumen and abomasal bloat	-	+
6	8	Emphysematous necrotic enterocolitis	+ A	+
7	8	Abomasal bloat and acute rumentitis	+ A	+
8	11	Abomasitis with perforating ulcer	+	-
9	8	Necrotic rumenitis, omasitis, abomasitis and ulcer	-	-
10	26	Emphysematous abomasitis, ulcer, subcutaneous emphysema	ND	+
11	7	Perforating abomasal ulcers	ND	-
12	7	Emphysematous abomasitis, necrotic enteritis	+ A	+
13	26	Abomasal bloat, abomasitis	-	+
14	12	Rumen and abomasal bloat, rumenitis	ND	+
15	12	Rumen and abomasal bloat, rumenitis	ND	+
16	8	Rumen and abomasal bloat, abomasitis	ND	+
17	7	Emphysematous abomasitis	+	+
18	7	Emphysematous abomasitis	+ A	-
19	7	Emphysematous abomasitis, enterotoxemia	+	+
20	9	Emphysematous abomasitis, typhlitis, omasitis, rumenitis, colitis	+	-
21	6	Emphysematous abomasitis, perforated ulcer	+	+
22	7	Emphysematous abomasitis, rumenitis, hemorrhagic enteritis	-	+
23	4	Emphysematous abomasitis, hemorrhagic enteritis, reticulitis	-	+
24	42	Abomasal bloat	+ A	+

The calves were all female and were all Holsteins except for calf 15 which was an Ayrshire.
ND = Not determined.
A = Type A *Clostridium perfringens* cultured.
+ = *C. perfringens* cultured but not typed.
- = *C. perfringens* not recovered.
* = The *Sarcina* positives were determined by histologic examination.
Post-mortem interval varied from 1 .5 h to 2 d, the latter under refrigeration.

believe that *C. perfringens* was responsible for the 24 deaths described here; however, it is difficult to understand the role of *Sarcina,* if any. These bacteria only occurred in the mucus and in the necrotic material at the surface, and were never seen in the mucosa or submucosa where gas was being formed. The *Sarcina* may have contributed to the bloat in some of the calves; however, 10 of the 17 in which *Sarcina* was demonstrated had no bloat. In the recent past, *Sarcina ventriculi* was cultured from the abomasal contents of 2 calves with abomasitis and bloat (3). Attempts to reproduce disease with *Sarcina* alone and with *Sarcina* plus *C. perfringens,* given by gavage to 4 dairy calves proved unsuccessful (Mills, personal communication, 2005). *Sarcina ventriculi* was also recovered from 1 of 29 cases of abomasal bloat in lambs, though the organism was seen in 12 of the 29 (4). The *Sarcina* spp. are difficult to cultivate, requiring sarcina growth medium, a pH of 2.2, and anaerobic conditions (4).

Emphysematous abomasitis has only rarely been documented, in a calf or lamb with *C. septicum* (5), a calf with *C. perfringens* infection (6), and in lambs with *C. fallax, C. sordellii,* and *Sarcina* (4). In the latter cases, the *Sarcina* clusters were notable, but localized to the contents on the surface, and not within the wall. Authors of a report of abomasal bloat in kid goats, accompanied by subcutaneous emphysema, regarded the *Sarcina* as the causative agent (7). Most recently, tympany, acidosis, and gastric emphysema were recognized in 1 dairy and 3 beef calves that died at 2 to 3 days of age (8). A *Clostridium* sp. was cultured from 1 calf, and *Sarcina* was seen in 3. Experimental calves fed milk replacer, corn starch, glucose, and warm water and dosed, by intubation, with 200 mL of rumen fluid from a cow reproduced colic and abdominal distention. At necropsy 3 of 5 studied had abomasal and ruminal edema and necrosis, and 2 had emphysematous gastritis. However, anaerobic cultures failed to recover *C. perfringens* from any of the 5, and *Sarcina* spp. were not reported either (8). There is a need for additional studies defining the roles of *C. perfringens, Sarcina,* and a combination of the 2 in disease in ruminant stomachs.

Type A *C. perfringens* appears to have been the cause of these acute abomasal deaths. It is remarkable that calves, which appeared normal at the time of the night feeding, would be dead with abomasal necrosis and bloat 12 h later. In those instances when clinical features were recognized, the acutely ill calves were depressed, bloated, ground their teeth, and refused to eat. They were seldom saved.

This manuscript documents that *C. perfringens* continues to be responsible for severe acute abomasal disease, particularly, as a consequence of what could be called over-management and inappropriate use of antibiotics rather than hygiene. Fecal contamination of colostrum may have been a key element. Without the farm visits, it appears unlikely that the disease described here would have remitted spontaneously. No new cases have occurred in 4 y.

Acknowledgment

The authors thank Dr. Radhakrishna Sura for assistance with the photographic images. CVJ

References

1. Roeder BL, Chengappa MM, Nagaraja TG, Avery TB, Kennedy GA. Isolation of *Clostridium perfringens* from neonatal calves with ruminal and abomasal tympany, abomasitis, and abomasal ulceration. J Am Vet Med Assoc 1987;190:1550–1555.
2. Roeder BL, Chengappa MM, Nagaraja TG, Avery TB, Kennedy GA. Experimental induction of abdominal tympany, abomasitis, and abomasal ulceration by interaruminal inoculation of *Clostridium perfringens* type A in neonatal calves. Am J Vet Res 1988;49:201–207.
3. Mills KW, Boerger-Fields A, McAllister MM. *Sarcina ventriculi:* Isolation and identification in abomasitis and bloat of neonatal calves. Proceedings, (abstr) 15th Annual West Food Anim Dis Res Conf, University of Nebraska, Lincoln Nebraska, March 1994.
4. Vatn S, Tranulis MA, Hofshagen M. *Sarcina*-like bacteria, *Clostridium fallax* and *Clostridium sordellii* in lambs with abomasal bloat, haemorrhage and ulcers. J Comp Path 2000;122:193–200.

5. Eustis SL, Bergeland ME. Suppurative abomasitis associated with *Clostridium septicum* infection. J Am Vet Med Assoc 1981;178: 732–734.
6. Songer JG, Miskimins DW. Clostridial abomasitis in calves: Case report and review of the literature. Anaerobe 2005;11:290–294.
7. DeBey BM, Blanchard PC, Durfee PT. Abomasal bloat associated with *Sarcina*-like bacteria in goat kids. J Am Vet Med Assoc 1996;209: 1468–1469.
8. Panciera RJ, Boileau MJ, Step DL. Tympany, acidosis, and mural emphysema of the stomach in calves: Report of cases and experimental induction. J Vet Diagn Invest 2007;19:392–395.

Answers to Quiz Corner
Les réponses du test éclair

1. **a)** *Eimeria* species are seldom pathogenic in pigs. Other parasites that can induce diarrhea in nursing pigs are *Cryptosporidium* and *Strongyloides* species.

a) *Eimeria* est rarement une espèce pathogène chez le porc. Les autres parasites qui peuvent provoquer une diarrhée chez les porcelets à l'allaitement sont *Cryptosporidium* et *Strongyloides*.

2. **b)** Respiration may cease with an incompatible transfusion; vomiting may occur when the rate of transfusion is excessively rapid.

b) La respiration peut arrêter lors d'une transfusion incompatible. Il peut y avoir des vomissements lorsque la vitesse de la transfusion est excessivement rapide.

3. **d)** This dog probably has acute pancreatitis, not pancreatic insufficiency. The latter is not commonly associated with acute pancreatitis. Ultrasonography (to rule out other diseases and confirm pancreatitis), nothing PO, and intravenous fluids are indicated in dogs with suspected pancreatitis.

d) Ce chien souffre probablement de pancréatite aiguë et non d'insuffisance pancréatique. Cette dernière n'est pas communément associée à une pancréatite aiguë. L'échographie (pour éliminer d'autres maladies et confirmer la pancréatite), rien per os et des liquides intraveineux sont indiqués chez les chiens chez qui on soupçonne une pancréatite.

4. **b)** Overly tight application of the tourniquet may damage the radial nerve.

b) L'application trop serrée d'un tourniquet peut endommager le nerf radial.

5. **e)** Dogs with uncomplicated diabetes mellitus are not hypocalcemic.

e) Les chiens souffrant de diabète sucré non compliqué ne présentent pas d'hypocalcémie.

6. **e)** *Mycobacterium paratuberculosis* infects the ileum and proximal large intestine.

e) *Mycobacterium paratuberculosis* infecte l'iléon et la portion proximale du gros intestin.

7. **a)** Grooming to remove crusts and bathing with an iodine-based shampoo are the best therapy for this disease.

a) Le brossage pour enlever les croûtes et des bains avec un shampooing à base d'iode sont le meilleur traitement pour cette maladie.

8. **c)** Administration of dexamethasone can precipitate laminitis.

c) L'administration de dexaméthasone peut accélérer la fourbure.

9. **b)** After intramammary administration of antibiotics, the milk from all four quarters must be discarded for the time indicated on the drug label, regardless of the number of quarters treated.

b) Après l'administration intramammaire d'antibiotiques, le lait des quatre quartiers doit être éliminé pour la période de temps indiquée sur l'étiquette du médicament, peu importe le nombre de quartiers traités.

10. **e)** Cirrhosis may result in reduced liver size. Determining the cause of the cirrhosis may allow you to arrest or reverse the process that initiated the disease and thereby help maintain the animal in remission.

e) Une cirrhose peut conduire à une diminution de la grosseur du foie. Le fait de déterminer la cause de la cirrhose peut permettre d'arrêter ou de renverser le processus qui a déclenché la maladie et dès lors aider à maintenir l'animal en rémission.