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Case Report

Outbreak of abomasal bloat in goat kids due to *Clostridium ventriculi* and *Clostridium perfringens* type A

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Abstract

We described an outbreak of abomasal bloat in goat kids. Increased mortality with a history of abdominal bloating, dullness, and death was reported. *Postmortem* exanimation revealed dehydration, pale mucosa, ascites, abomasal tympanism and intestinal meteorism and congestion, and emphysematous abomasitis. Cytological evaluation from abomasum revealed gram-positive bacteria with a cuboid shape suggestive of *Clostridium ventriculi*, gram-positive bacilli suggestive of *Clostridium perfringens*, and ovoid basophilic yeasts. *C. ventriculi* and *C. perfringens* type A were confirmed by anaerobic culture and molecular tests. Histopathological findings revealed emphysematous abomasitis, necrosis of the gastric and intestinal walls, gastroenteritis, and intestinal thromboembolism. The possible associated factor was erroneous use of the milk replacer associated with inadequate kid management. Finally, the prophylactic measures suggested such as hygiene care, proper use of milk replacer, clostridial vaccination plan, and a good colostrum management were able to control the outbreak.

Key words: artificial feeding, *Clostridium* spp., enterotoxemia, milk replacement.

An outbreak of abomasal bloat occurred in a dairy goat farm located in Rio de Janeiro, Brazil, with 650 Saanen goats raised under intensive husbandry management. In late spring (November 2017), during mid-kidding season, increased mortality in suckling goat kids from 1 to 3 months old was reported. A sudden acute condition that involved abdominal bloating, dullness, and death within 6–12 h was noted. Likewise, 20.0% (11/55) of the kids born so far had died with the same clinical signs. At the first clinical examination, sick kids showed distension of the abdomen, a fair amount of content in the abomasum by ballottement, distension of the right and left paralumbar fossa based on the presence of gas on percussion, dullness, anorexia, and dehydration.

An epidemiological survey was conducted to verify the possible associated factors related to the disorder. The morbidity, fatality, and mortality rates were calculated from dairy farm data 45 days after the first technical visit and before application of corrective measures (applied at the second visit). In addition, the frequencies of deaths due to acute or relapsed clinical features, as well as the frequencies of sick animals and relapses without death were assessed. Relapses were considered as ≥2 days without the previous feature of abomasal bloat.

Among all of the necropsied animals (n = 11; not the same kids described above), seven (Cases 1–7) with previous history of abomasal bloat presented mainly gastrointestinal findings with dehydration, pale mucosa, ascites (reddish content), abomasal tympanism and intestinal (mainly caecum) meteorism and congestion, emphysematous abomasitis (four of them with abomasal rupture and content in the cavity; peritonitis), blood clots inside the left ventricle, renal congestion and cranial areas of lung consolidation (Fig. 1-4). The other four (Cases 8–11; control ones) with no history of

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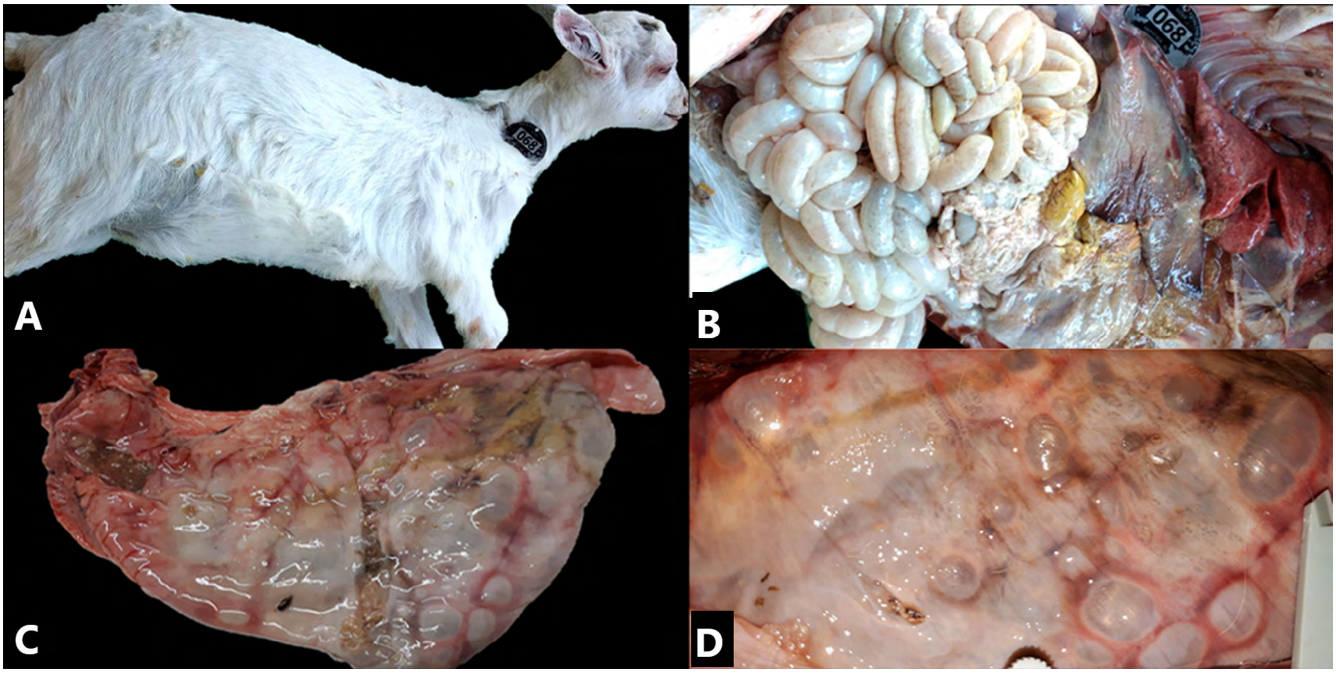

Figure 1. Kid goat with 45 days of age,case 3. **A.** It was found dead with intense abdominal bloating and subcutaneous emphysema. **B.** Free abomasal contents in the abdominal cavity and peritonitis. **C.** Abomasal congestion and diffuse emphysema in the abomasal wall. **D.** Details of the emphysema in the abomasal wall.

Table 1. Overall results obtained from molecular biology, bacteriology, and cytology data obtained from the outbreak of abomasal bloat in suckling kid Saanen goats (affected ones, Cases 1–7; control ones, Cases 8–11) raised under intensive management.

Cases (n=11)	PCR		Bacteria Culture	*Differential Staining*	
	PDC gene	16S rRNA gene	Isolation and molecular id.	GRAM	Romanowsky
Case 1	Positive	Positive	*Clostridium perfringens* type A	S-	C+++ / L+++
Case 2	Negative	Positive	*Clostridium perfringens* type A	S-	C+++ / L+++ / Small bacillus
Case 3	Positive	Positive	*Clostridium perfringens* type A	S-	C+++ / L+++
Case 4	Positive	Positive	Negative	S-	C+++ / L+ / Small Bacillus
Case 5	Positive	Positive	*Clostridium perfringens* type A	S-	C+++ / S++ / L +++ / Small Bacillus
Case 6	Positive	Negative	*Clostridium perfringens* type A	S+++	C++ / L+++
Case 7	Negative	Negative	Negative	S+	C +++ / L+ / Small Bacillus
Case 8	Negative	Positive	*Clostridium perfringens* type A	S-	Negative / Inflammatory infiltrate
Case 9	Negative	Positive	Negative	S+	Negative / Inflammatory infiltrate
Case 10	Negative	Positive	Negative	S+	Negative
Case 11	Negative	Negative	Negative	S+	Negative

Environment (n=7)	PDC gene	16S rRNA gene
Shavings of nursery	Negative	Positive
Concentrate from feeder	Negative	Positive
Concentrate from packing (kid goats)	Negative	Positive
Concentrate from packing (Lactation goats)	Negative	Positive
Concentrate from packing (pre-partum)	Negative	Positive
Rabbit feces	Negative	Positive
Kidfold feces	Negative	Positive

Abbreviations: S, gram-positive bacteria, coccoid, with a cuboid shape suggestive of *C. ventriculi*; C, gram-positive bacilli, forming oval-subterminal spores suggestive of *C. perfringens*; L, round-to-ovoid basophilic yeasts compatible with *S. cerevisiae*.

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abomasal bloat presented mainly respiratory findings with dehydration, pulmonary consolidation and edema, pleuritis, foam in the airways, and blood clots inside left ventricle. Both groups were separated in Table 1 to verify results from other complementary findings.

From all postmortem examinated animals, the following were collected: (a) agar gel transport swabs (Thermo Scientific) from the abomasal mucosa surface, (b) abomasal content in microtubes (2 mL), and (c) fragments of abomasal tissue in microtubes (2 mL) for bacteriological culture and typification. For molecular detection, the fragments of the abomasal tissue and seven environment samples from the concentrate offered to the kids, feces from the sick animals, and feces from the rabbits that were kept near the kidding sector were also collected. For *C. perfringens* detection, samples were pre-enriched on the Tarozzi medium and then streaked onto Schaedler agar with 5% sheep blood incubated under anaerobic conditions. Then, suspected colonies were subjected to multiplex polymerase chain reactions for confirmation and typification (11). For the molecular detection of *Clostridium ventriculi*, DNA extraction from the samples collected was realized using a commercial kit (EpicentreT), following the manufacturer's instructions. Conventional polymerase chain reaction was used to detect *C. ventriculi* using primers from the pyruvate decarboxylase (PDC) gene (specific to *C. ventriculi*) and the 16S rRNA gene (used to detect *Clostridium* sp.), according to Lam-Himlin et al. (9). The results obtained from molecular biology, bacteriology data, and environment samples were summarized in Table 1.

From all postmortem examinated animals (n = 11), multiple organ samples were collected and fixed in 10% buffered formalin. The tissue samples were routinely

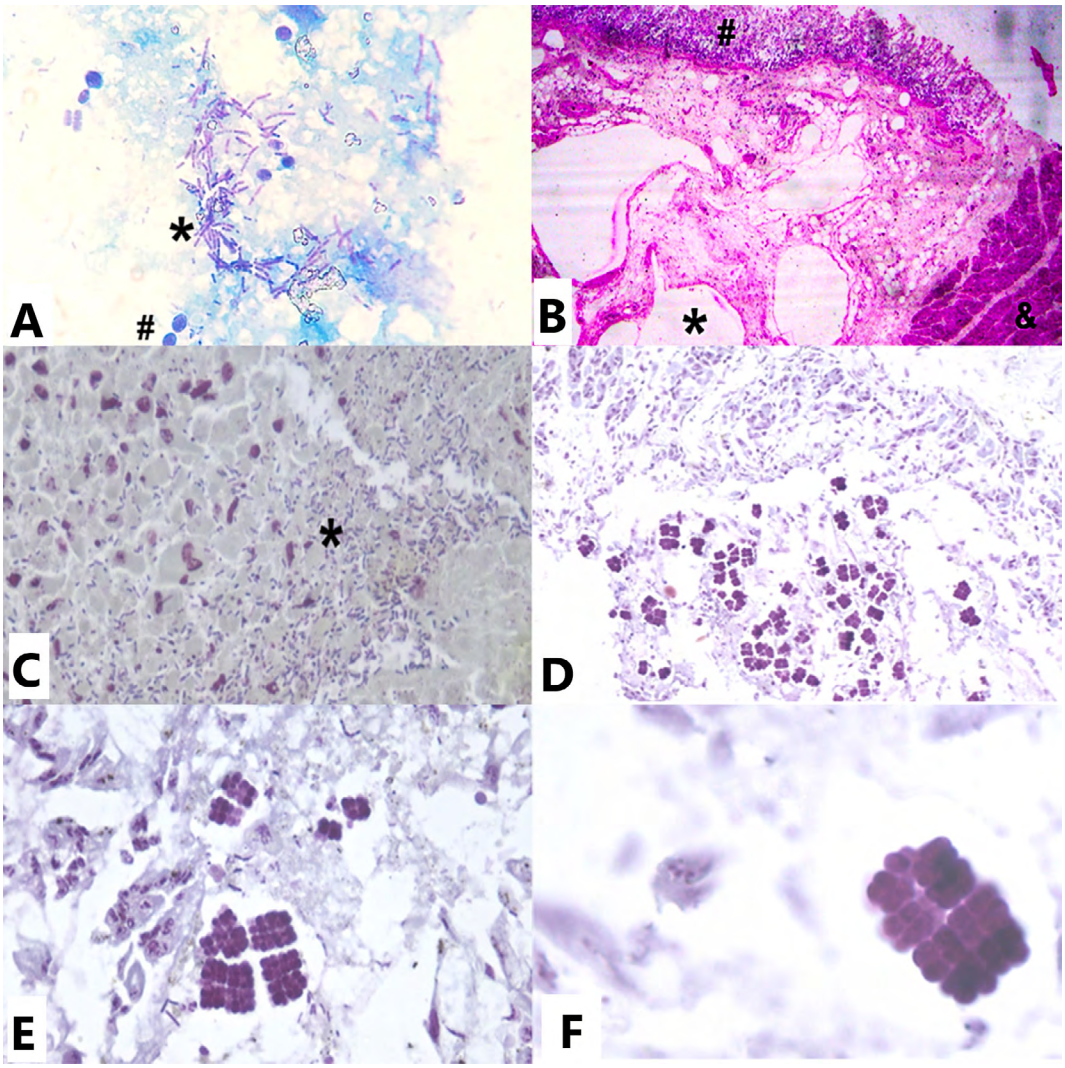

Figure 2. Photomicrograph of abomasal tissue.A.Imprinting of abomasal mucosa - *C. perfringens* characterized as basophilic rods (*) and round to ovoid yeasts (#; 5–10 µm). Rapid Panoptic Staining. B.(a) Emphysematous abomasitis – gas accumulated at the mucosa (#), submucosa (*), and muscle tunics (&) of the abomasum. H&E staining (500 µm). C.Necrosis of the abomasal wall with *C. perfringens* characterized as basophilic rods (Gram+; *).D, E and F. (8, 100 µm; 9, 50 µm; 10, 20 µm) *C. ventriculi* in the abomasal mucosa in different magnifications. Gram staining.

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processed and stained with hematoxylin and eosin and Gram stains (8). Direct impressions of the abomasal mucosa were made using glass slides (6). Slides were made in triplicate for each animal (n = 11), air-dried, and stained using three different techniques, GIEMSA, Quick Panotic, and Gram stains (8) for cytological evaluation.

Through all cytological evaluations, it was possible to identify the following: (a) gram-positive bacteria, coccoid, with a cuboid shape suggestive of *C. ventriculi* (Fig. 8-10); (b) gram-positive bacilli, forming oval-subterminal spores suggestive of *C. perfringens* (Fig. 5); (c) round-to-ovoid basophilic yeasts (Fig. 5); and (d) a scarce population of small basophilic bacilli and streptobacilli (Table 1).

Kid goats with a history of abomasal bloat (n = 7; Cases 1–7) and gastroenteritis presented at histopathology: cholangiohepatitis, coagulation necrosis of renal contorted tubules, emphysematous abomasitis (necrosis and emphysema in the abomasal wall; Fig. 6-7), intestinal necrosis, thromboembolism, pulmonary edema and non-purulent pneumonia. Of those without signs of abomasal bloat (n = 4; control group; Cases 8-11) and respiratory findings, histopathology showed purulent bronchopneumonia, coagulation necrosis of renal contorted tubules, and intestinal necrosis with a mixed picture of mannheimiosis and enterotoxemia.

Goat kids with clinical signs of abomasal bloat and apathy were treated with 30,000 IU/kg of benzathine benzylpenicillin along with 120.0 mg/kg of dihydrostreptomycin (Agrovet Plus; Novartis) and 2.2 mg/kg of flunixin meglumine (Niglumine; Ceva) intramuscularly once per day for 3 consecutive days. After the treatment onset, in about 6–12 h, improvement (appetite and reduction of the abdominal contour) or death of the animal was verified, corroborating the acute clinical course of the disease.

Also, after birth, the kids were immediately separated from their mothers and received 200 mL of pasteurized colostrum, twice a day, for up to 2 days. From the age of 2 to 10 days, they were transitioned from goat's milk to milk replacer [20% protein, 18% fat, and probiotic *Saccharomyces cerevisiae* (Cabra Milk, Repamix)] providing around 25% of the kid's live weight/day divided into three equal portions, offered during the day. Milk replacer was then used until 90 days of age. From 15 days of age, concentrates and water were offered *ad libitum*. The main possible associated factors and preventive measures used to control the outbreak are indicated in Table 2.

From dairy farm data collection (45 days between two visits), the morbidity, mortality, and fatality rates were 53.7% (44/82), 15.9% (13/82), and 29.5% (13/44), respectively. A total of 76.9% (10/13) of kids that died developed an acute disease course (<18 h) and other three kids showed two (2/13) or three (1/13) relapses before death. From recovered sick kids, 51.6% (16/31) did not present posterior relapse and 22.6% (7/31), 16.1% (5/31), and 9.7% (3/31) showed one, two, or more than three relapses, respectively. Lastly, considering a total of 82 births (up to corrective measures), with 13 deaths during data collection and seven deaths before due to abomasal bloat, the final mortality rate was 24.4% (20/82).

According to Panciera *et al.* (12) and Burgstaller et al. (3), abomasal bloat syndrome can be multifactorial, and the pathophysiology primarily involves excessive fermentation of high-energy gastrointestinal contents in the abomasum (from milk, milk replacer, or high-energy OES), as well as bacterial activity releasing fermentative enzymes leading to gas production and bloating. Regarding the bacterial etiology, in agreement with the current report, the most frequently isolated bacterial pathogens include *Clostridium perfringens* type A and *Clostridium ventriculi* (formerly *Sarcina ventriculi)* (4,5,10,18,19). Additional bacterial pathogens isolated from calves with abomasal bloat include α *Streptococci*, other *Streptococci* spp., *Escherichia coli, Clostridium fallax, and Clostridium sordellii* (15,19). The pathogenic role of *C. perfringens*

Table 2. Possible associated factors found in the management of sucking kid goats in the dairy goat farm along with suggestions made for correction and prevention.

Associated factors	Suggestion for correction or risk reduction
Milk substitute prepared and offered at 40ºC	Milk substitute prepared and offered at 25ºC
Large volume of milk per meal (Almost 25% live weight/day)	Supply 15% of live weight/day divided 3 times a day
Heterogeneous mobs with older kid goats that can drink faster than younger ones.	Segregate lots of kid goats by weight and size, for the purpose of calculating the necessary volume of milk to feed the kid goats and to reduce unequal competition.
Young goats were only given one to 2 minutes to drink from the feeding bottle. After that, they were not full, so they went and ate the concentrate.	Restrict access to the concentrate until 2 hours after giving the milk bottle.
Overcrowding	Recalculate by available area for each mob (kids) or organize the farrowing station to respect the capacity of goats.
Possible failure of the immunization program and absence of a colostrum quality plan	Vaccination plan containing *C. perfringens* alpha toxoid and establish a good colostrum quality plan

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type A was first demonstrated by intraruminal inoculation of such bacteria in neonatal calves, resulting in anorexia, depression, abomasal bloat, diarrhea, and death (14). Interestingly, this agent has also been recently reported in cases of gas gangrene in cattle (13). Lastly, Khan et al. (7) reported a greater prevalence of pathogenic *C. perfringens* type A in goat kids than types B and D.

The role of *C. ventriculi* as a primary agent in gastrointestinal disorders is well-described in humans (17), even though it is also considered a normal commensal microbiota found in the control kids (Cases 8–11) from the current report. Therefore, these bacteria can be considered opportunistic when the environment becomes conducive to its growth (2). Although the pathogenic role of *C. ventriculi* is not clear, the local accumulation of acetaldehyde and ethanol formed from carbohydrate fermentation by the organism could induce stomach and duodenal injuries. Also, the *C. ventriculi* detection was possible by molecular or histology/cytology methods, since this agent is hardly detected by classical culture techniques (4,5,19). Equally, its detection must be associated with pathological findings, as such finding by chance does not point toward a clinical risk to the animal.

S. cerevisiae fermentation products have been extensively used in the dairy industry with beneficial effects on production parameters (2). Although there are several positive arguments regarding the introduction of such yeast into the ruminant diet, disturbances in gastric emptying associated with milk accumulation may have provided an opportunistic and deleterious growth of *S. cerevisiae* within the kids' abomasum. They also ferment sugars, releasing ethyl alcohol and carbon dioxide, similar to *C. ventriculi*. Therefore, as the authors found a great amount of yeasts in the kids affected by abomasal bloat, attention should be paid when adding yeasts to the diet of young ruminants.

Regarding the possible associated factors with the occurrence of abomasal bloat, our results are in agreement with the findings from most worldwide reports, that is, high infection rates were related to microbiota imbalances and poor hygiene. All environment/food samples (7/7) collected in this case were positive for the 16S rRNA gene related to *Clostridium* spp. Likewise, the use of a milk substitute instead of natural goat milk can be closely related to the disturbance in abomasal emptying. Large volume of milk ingested in a short time, high osmolarity, and high temperature of the milk replacer associated with kid management are other strong reasons verified in previous reports (2,4,18). Moreover, the offering of *ad libitum* concentrate to the kids and their behavior to look for such food immediately after milking may also have favored the risk of enterotoxemia due to a great amount of milk and soluble carbohydrates within the abomasum.

Regarding prophylaxis, the high frequency of *C. perfringens* type A detection in several reports on abomasal bloat and gas gangrene in ruminants reinforces that such an agent should be part of the routine clostridial vaccines. Therefore, it is important to look for this information at the time of purchase and immunization of the flock. In the current report, pregnant goats were vaccinated with a commercial vaccine containing alpha toxin, so it is believed that since there was no colostrum management program, the goat kids could be receiving low quality colostrum and immunization was not efficient. In this sense, the importance of quality plans for colostrum management in dairy farms is also emphasized.

Lastly, few reports on the treatment of abomasal bloat have been described. Most therapies included antibiotics (primarily penicillin and ampicillin), rumen "tonics" (including a wide variety of medicaments), nonsteroidal anti-inflammatory drugs (primarily flunixin meglumine), relieving the distension via a tube or trocar, clostridial antitoxin, and fluid therapy (16). In the authors' experience, advanced cases did not respond to antibiotic therapy and anti-inflammatory drugs. Therefore, early detection of sick kids and quick initiation of treatment were the main determinants of the prognosis of each case.

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Conflict of Interest Statement

The authors declared no potential conflicts of interest in relation to the research, authorship or publication of this article.

Authors' Contribution

MFAB was the lead clinician, collected the data on farm, performed necropsies, collected biological samples for postmortem evaluation, and drafted and revised the manuscript. FMG, FSCL, and IOC were assisting clinicians and assisted with all clinical inspections, necropsies, and collection of biological samples and revised the manuscript. NCC was the lead molecular biologist and performed all the molecular evaluations and drafted and revised the manuscript. JAV and NXS were undergraduate vet students who helped with molecular evaluations and revised the manuscript. FZB assisted in the critical revision of the manuscript. SM and AFCN were the lead microbiologists and performed all the bacteriological evaluations and drafted and revised the manuscript. CDF was the lead pathologist and performed all the histopathology and cytological examinations and drafted and revised the manuscript.

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