

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

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June 1, 2020

Abstract

This study aimed to describe an outbreak of abomasal bloat in kid goats and its clinical, pathological, microbiological, molecular, and epidemiological characteristics. In the kidding season, increased mortality of kid goats with a history of abdominal bloating, dullness, and death was reported. Clinical examinations were carried out, and biological samples from necropsied kids (n = 11) were collected for pathological, microbiological, and molecular diagnosis. Likewise, an epidemiological survey was carried out in order to verify possible associated factors related to the disorder. A therapeutic protocol was also implemented. The main necropsy findings were dehydration, pale mucosa, ascites, abomasal and intestinal meteorism and congestion, emphysematous abomasitis, and cranial areas of lung consolidation. Through staining techniques for cytological evaluations of the abomasum, it was possible to identify Gram positive bacteria, coccoid, with a cuboid shape suggestive of *Clostridium ventriculi*, Gram positive bacilli suggestive of *Clostridium perfringens* and ovoid basophilic yeasts compatible with *Saccharomyces cerevisiae*. By anaerobic culture and molecular tests, *C. ventriculi* and *C. perfringens* type A were confirmed. The main histopathological findings were cholangiohepatitis, nephrosis, emphysematous abomasitis, hyalinization of the gastric and intestinal walls, gastroenteritis, intestinal thromboembolism, pulmonary edema, and non-purulent pneumonia, overall suggesting a systemic enterotoxemia picture. The early detection of sick kids and quick initiation of treatment were the primary determinants of the prognosis of each case. There was a final mortality rate of 24.4% (20/82), and the agents *C. perfringens* type A and *C. ventriculi* were identified as the main ones involved, with the possible participation of *S. cerevisiae*. Among the possible associated factors, the erroneous use of the milk replacer associated with inadequate kid management was verified. Among the prophylactic measures, hygiene care, proper use of milk replacer, vaccination plan containing *C. perfringens* alpha toxoid associated with a good colostrum management were suggested.

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Outbreak of abomasal bloat in goat kids

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SUMMARY

This study aimed to describe an outbreak of abomasal bloat in kid goats and its clinical, pathological, microbiological, molecular, and epidemiological characteristics. In the kidding season, increased mortality of kid goats with a history of abdominal bloating, dullness, and death was reported. Clinical examinations were carried out, and biological samples from necropsied kids ($n = 11$) were collected for pathological, microbiological, and molecular diagnosis. Likewise, an epidemiological survey was carried out in order to verify possible associated factors related to the disorder. A therapeutic protocol was also implemented. The main necropsy findings were dehydration, pale mucosa, ascites, abomasal and intestinal meteorism and congestion, emphysematous abomasitis, and cranial areas of lung consolidation. Through staining techniques for cytological evaluations of the abomasum, it was possible to identify Gram positive bacteria, coccoid, with a cuboid shape suggestive of *Clostridium ventriculi*, Gram positive bacilli suggestive of *Clostridium perfringens* and ovoid basophilic yeasts compatible with *Saccharomyces cerevisiae*. By anaerobic culture and molecular tests, *C. ventriculi* and *C. perfringens* type A were confirmed. The main histopathological findings were cholangiohepatitis, nephrosis, emphysematous abomasitis, hyalinization of the gastric and intestinal walls, gastroenteritis, intestinal thromboembolism, pulmonary edema, and non-purulent pneumonia, overall suggesting a systemic enterotoxemia picture. The early detection of sick kids and quick initiation of treatment were the primary determinants of the prognosis of each case. There was a final mortality rate of 24.4% (20/82), and the agents *C. perfringens* type A and *C. ventriculi* were identified as the main ones involved, with the possible participation of *S. cerevisiae*. Among the possible associated factors, the erroneous use of the milk replacer associated with inadequate kid management was verified. Among the prophylactic measures, hygiene care, proper use of milk replacer, vaccination plan containing *C. perfringens* alpha toxoid associated with a good colostrum management were suggested.

KEYWORDS: Artificial feeding, goats, *Clostridium* spp., enterotoxemia, milk replacement.

1| INTRODUCTION

Alternative rearing systems are a high priority for dairy goat farmers. Suckling kid goats can be reared using milk replacers, and weaned at an early age, six weeks or less, if adequately mature. The advantages of milk replacers include reduced production costs, increased production, and breakage of disease cycles caused by small ruminant lentiviruses (Konishi et al., 2011). However, some farmers believe that kids reared with milk replacers provide tougher meat and are opposed to this practice. This belief could be explained by the fact that most of the kid meat with high pH comes from kids raised on milk replacers, which might induce tough meat (Ripoll et al., 2019). The success of rearing kids using milk replacers requires strict adherence to correct management practices, particularly in ensuring good hygiene in rearing facilities, and cleanliness of feed and feeding equipment (Mowlem, 1984).

Products and technologies including novel milk replacers, automated goat feeding systems, and the acidification of the milk, have all been introduced to assist dairy farmers who wish to feed for improving pre-weaning growth. Some of these improvements of feeding have the potential to alter abomasal emptying, which refers to the time the chymus remains in the abomasum before passing into the intestinal tract (Burgstaller et al., 2017). When the management feeding practice is not correct, it can significantly prolong abomasal emptying and increase rates of gastrointestinal diseases in young ruminants, such as abomasal bloat (DeBey et al., 1996; Songer and Miskimins, 2005; Van Kruiningen et al., 2009; Leite Filho et al., 2016). Abomasal bloat associated with *Clostridium ventriculi* is a rare disease occasionally reported in young ruminants (Edwards et al., 2008). This syndrome may be due to excess carbohydrate fermentation in the abomasum by

gas-producing bacteria in the abomasum (such as the *Lactobacillus* or *Clostridium* genus) (Panciera et al., 2007).

Clostridium ventriculi and *Clostridium perfringens* Type A have been associated with many gastric disorders, such as in cases of delayed gastric emptying and gastric outlet obstruction. *Clostridium ventriculi* has been observed in lambs with acute dilatation of the abomasum (Vatn et al., 2000; Edwards et al., 2008) and has been reported in association with abomasal bloat in calves and kids (DeBey et al., 1996).

These bacteria are distinguished by exhibiting cubical arrangement groups, often in tetrads, and occasionally in cubes formed by eight cells (Canale-Parola, 1970). They are ubiquitous, coccoid, Gram positive obligate anaerobes, and were first documented in the human gastrointestinal tract (Goodsir and Wilson, 1842). Several types of *C. perfringens* have been categorized on the basis of toxin production. *Clostridium perfringens* type A, producing α toxin, is a common isolate from cases of caprine enterotoxemia (Miyashiro et al., 2007), even though its pathologic role is equivocal as it is a normal commensal of the gastrointestinal tract (Uzal et al., 2010). In this context, this study aimed to describe an outbreak of abomasal bloat in kid goats and its clinical, pathological, microbiological, molecular, and epidemiological characteristics.

2| MATERIALS AND METHODS

2.1| CLINICAL HISTORY AND EPIDEMIOLOGICAL DATA

The outbreak 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), in the mid-kidding season, increased mortality in suckling kid goats among one and three months old was reported by the farmer. The history was a sudden acute condition that involved abdominal bloating, dullness, and death within 6 to 12 hours. Likewise, 20.0% (11/55) of the kid goats born so far had died with the same clinical signs. At the first clinical examination, sick kid goats showed a distended abdomen, a fair amount of content in the abomasum by ballottement, right and left paralombar fossa distended by the presence of gas in the percussion, dullness, anorexia, and dehydration (Figure 1 A and B). From seven animals that died with clinical signs of abomasal bloat and other four that died without such symptoms (classified as control group), biological materials were collected for pathological, microbiological, and molecular diagnosis, as will be described in sequence.

Likewise, an epidemiological survey was carried out in order to verify the possible associated factors related to the disorder. A descriptive observational study represented by descriptive and intervention phases were adopted to develop control and prevention measures (Stevenson et al., 2017). Morbidity, lethality and mortality rates were also calculated from dairy farm data based on the 45 days subsequent to the first technical visit and before management corrective measures (At the second visit time). In addition, frequencies of deaths due to acute or relapses clinical features, as well as frequencies of sick animals and relapses without death were assessed. Relapses were considered as two or more days without the previous feature of abomasal bloat.

2.2| PATHOLOGICAL AND CYTOLOGICAL EVALUATION

From all necropsied animals ($n = 11$), multiple organ samples were collected and fixed in 10% buffered formalin. The material was sent to the Laboratory of Pathological Anatomy of the Biological Institute in São Paulo State. The tissue samples were routinely processed, embedded in paraffin, and sections were stained with hematoxylin and eosin (HE) and Gram (Kiernan, 2008; Yen et al., 2019).

Since the main pathological findings were located in the abomasum, direct impressions of the abomasal mucosa were made using glass slides (Kamatchi et al., 2015). Slides were made in triplicate for each animal ($n = 11$), air-dried, and stained by three different techniques, GIEMSA, Quick Panotic, and Gram (Merck KGaA, 2014) for cytological evaluation under optical microscopy.

2.3| BACTERIOLOGICAL AND MOLECULAR EVALUATION

Since the main pathological findings were located in the abomasum, from all necropsied animals ($n = 11$) were collected: (a) agar Gel Transport Swabs (Thermo Scientific) from the abomasal mucosa surface, (b)

abomasal content in microtubes (2 mL; Eppendorf), and (c) fragments of abomasal tissue in microtubes (2 mL; Eppendorf) for bacteriological culture and typification. For molecular diagnosis, it were also collected fragments of abomasal tissue and seven environment samples from the concentrate offered to the kid goats, feces from the sick animals, and feces from the rabbits that were kept near the kidding sector.

For *C. perfringens* diagnosis, samples were pre-enriched on Tarozi medium and then streaked onto Schaedler agar with 5% sheep blood incubated under anaerobe conditions. Then, suspected colonies were submitted to multiplex PCR reactions for confirmation and typification (Meer and Songer, 1997).

For molecular diagnosis of *Clostridium ventriculi*, DNA extraction from the samples collected was realized by use of a commercial kit (Epicentre™) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was used to diagnose *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. (2011). A negative control containing ultra-pure water was included in the reaction.

Amplicons were visualized under UV light after electrophoresis on 1.5% agarose gels stained by GelRed.

2.4| THERAPEUTIC PROTOCOL

Kid goats with clinical signs of abomasal bloat and apathy were treated with 30.000 UI/kg of benzathine benzylpenicillin along with 120.0 mg/kg of dihydrostreptomycin (Agrovet Plus; Novartis, Sao Paulo, Brazil) and 2.2 mg/kg of flunixin meglumine (Niglumine; Ceva, Sao Paulo, Brazil) IM SID for three consecutive days.

3| RESULTS

3.1| EPIDEMIOLOGICAL FINDINGS

Regarding the breeding system and environment, the kid barn contained six collective 2 m x 1 m kid pens (for different ages) with common access to a solarium area (Figure 1D). The pens had a concrete floor covered with shavings. Lateral walls were 1.5 meters high without windows with poor ventilation and they were overcrowded (Figure 1C). After birth, the kids were immediately separated from their mothers and they received 200 mL of pasteurized colostrum, twice a day, up to two days old. From two to ten days old, they received transition goat's milk as the milk replacer (20% protein, 18% fat content and probiotic *S. cerevisiae* (Cabra Milk, Repamix(r), Sao Paulo, Brazil) providing around 25% of the kid's live weight/day divided into 3 equal portions, offered during the day. Milk replacer was then used until weaning at 90 days old. Likewise, from 15 days old, concentrates and water were offered *ad libitum*.

Regarding feeding management, the milk replacer was prepared locally at a proportion of 120 g/1 L of water (recommended by the manufacturer), mixing with water at 40°C until it was homogeneous. The milk replacer was offered inside a plastic bottle, previously cleaned with boiled water, to feed the kids. The bottle was filled with 500 mL of milk replacer and then offered to the kids using a bottle support present at site. All sucking times, carried out with heterogeneous kid mobs, lasted for up to two minutes per mob, and then some kids began eating the concentrate.

Regarding the immunization program, pregnant goats received a single dose of vaccine against clostridiosis containing alpha toxoid (Covexin 9; MSD Saude Animal Brasil; Sao Paulo, Brazil), pasteurellosis, and colibacillosis (Paraven; Vencofarma, Sao Paulo, Brazil) at 30 days before delivery. Female and male kids received their first doses against clostridiosis (Covexin 9; MSD Saude Animal Brasil; Sao Paulo, Brazil) at one and two months old. In summary, the main possible associated factors and preventive measures indicated are summarized in Table 1.

From dairy farm data collection (45 days), the morbidity, mortality and lethality 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, during this period, developed an acute disease course (less than 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 presented 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 (45 days) and seven deaths before due to the abomasal bloat, the final mortality rate was 24.4% (20/82).

3.2| PATHOLOGICAL AND CYTOLOGICAL FINDINGS

Among all of the necropsied animals ($n = 11$), seven with previous history of abomasal bloat presented mainly gastrointestinal findings with dehydration, pale mucosa, ascites (reddish content), abomasal and intestinal (mainly caecum) meteorism and congestion, emphysematous abomasitis (some with ruptures and abomasal content in the cavity), blood clots inside the left ventricle, renal congestion and cranial areas of lung consolidation (Figure 2). The other four with no history of abomasal bloat presented mainly respiratory findings with dehydration, pulmonary consolidation and edema, pleuritis, foam in the airways, and blood clots inside left ventricle. The two groups are separated in Table 2 in order to verify results from other complementary findings.

Through all staining techniques (GIEMSA, Quick Panotic and Gram) prepared for cytological evaluations of the direct impressions from the abomasum, it was possible to identify, in different quantities: (a) Gram positive bacteria, coccoid, with a cuboid shape suggestive of *C. ventriculi*; (b) Gram positive bacilli, forming oval-subterminal spores suggestive of *C. perfringens*; (c) round to ovoid basophilic yeasts compatible with *S. cerevisiae*; and (d) a scarce population of small basophilic bacilli and streptobacilli (Figure 3; Table 2).

The main histopathological findings from the kid goats with history of abomasal bloat ($n = 7$) and gastroenteritis characterized by necropsy were: cholangiohepatitis, nephrosis, emphysematous abomasitis (hyaline necrosis and emphysema in the abomasal wall), intestinal hyaline necrosis, thromboembolism, pulmonary edema, and non-purulent pneumonia characterizing a systemic enterotoxemia picture. From the kid goats without abomasal bloat signs ($n = 4$; control group) and respiratory findings characterized by necropsy, it were found: purulent bronchopneumonia, nephrosis, and intestinal hyaline necrosis featuring a mixed picture of mannheimiosis and enterotoxemia (Table 2).

3.3| BACTERIOLOGICAL AND MOLECULAR FINDINGS

From the anaerobic culture and molecular identification, 71.4% (5/7) of kids with abomasal bloat signs were positive for *C. perfringens* type A. Also, 25.0% (1/4) of kids without abomasal bloat signs were positive for *C. perfringens* type A as seen in Table 2. Regarding the molecular analysis, a total of 71.4% (5/7) of kids with abomasal bloat signs were positive for both sets of primers (PDC and the 16S rRNA genes). Likewise, 0.0% (0/4) and 75.0% (3/4) of kids without abomasal bloat signs were positive for PDC and 16S rRNA genes, respectively. From the environment samples ($n = 7$), all were negative for the PDC primer (specific to *C. ventriculi*), and 100.0% (7/7) were positive for the 16S rRNA primer used to detect *Clostridium* sp. (Table 2).

3.4| THERAPEUTIC PROTOCOL

After the treatment onset, in about 6–12 h, the improvement (appetite and reduction of the abdominal contour) or death of the animal was verified, corroborating the acute clinical course of the disease. Absolute numbers regarding the effectiveness of the stipulated treatment are presented in morbidity, mortality and lethality rates described previously. Also, it is highlighted that as soon as treatment began from detection of clinical signs, a better kid's response to the treatment and survived were reached. 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.

4| DISCUSSION

To the best of the authors' knowledge, this is the first description of an outbreak of abomasal bloat (emphysematous abomasitis) in a dairy goat flock in Latin America. Likewise, based on an epidemiological investigation combined with pathological, microbiological, and molecular diagnosis, it was possible to identify the agents and associated factors involved. Therefore, we emphasize the importance of a broad diagnostic approach in order to elucidate and resolve health conflicts within rural establishments.

According to Panciera *et al.* (2007) and reviewed by Burgstaller, Wittek, and Smith (2017), abomasal bloat syndrome can be multifactorial, and the pathophysiology primarily involves excess 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 bloat. In addition, such a progression would also be enhanced by any factor that slowed abomasal emptying or caused paralytic ileus. Regarding the bacterial etiology, in agreement with the current report, the most frequently isolated bacterial pathogens include *C. perfringens* type A and *C. ventriculi* species (DeBey *et al.*, 1996; Vatn *et al.*, 2000; Edwards *et al.*, 2008; Van Kruiningen *et al.*, 2009; Leite Filho *et al.*, 2016). Additional bacterial pathogens isolated from calves affected with abomasal bloat include α streptococci, other streptococci species, *E. coli*, *C. fallax*, and *C. sordellii* (Roeder *et al.*, 1987; Vatn *et al.*, 2000).

The pathogenic role of *C. perfringens* type A was first demonstrated by intraruminal inoculation of such bacteria in neonatal calves, resulting in anorexia, depression, abomasal bloat, diarrhea, and death (Roeder *et al.*, 1988). Clinical severity is associated with the production of lethal toxins as the major cause, namely alpha (CPA; necrotizing and hemolytic action), although other toxins such as enterotoxin (CPE; enterotoxic), beta2 toxin (CPB2; enterotoxic and cytotoxic) and beta-like toxin (NetB; enterotoxic and cytotoxic) can also be produced by this agent (Freedman *et al.*, 2015). Interestingly, this agent has also been reported in cases of gas gangrene in horses and, more recently, in cattle (Peek *et al.*, 2003; Pires *et al.*, 2017). Lastly, Khan *et al.* (2020) reported a greater prevalence of pathogenic *C. perfringens* type A in kid goats when compared to B and D types.

While *C. perfringens* has been associated with food poisoning in humans, the role of *C. ventriculi* as a primary agent in gastrointestinal disorders (e.g., emphysematous gastritis) is well-described in humans (Freedman *et al.*, 2015; de Meij *et al.*, 2017; Singh, 2019). In addition, it is found in patients with gastric ulcers and delayed gastric emptying, but it is also considered a normal commensal microbiota, and it was also found in our control kids in the current report. Therefore, it is considered to be opportunistic when the environment becomes conducive to its growth (Crowther, 1971; Al Rasheed and Senseng, 2016). 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. Furthermore, the carbon dioxide production from glucose fermentation and pyruvate metabolism results in abdominal distention, as previously reported in some human patients (Tolentino *et al.*, 2003).

Regarding the diagnosis, confirmation of *C. ventriculi* infection is feasible by molecular or histology/cytology methods, since this microorganism cannot be detected by classical culture techniques, which in most cases reveal negative results (DeBey *et al.*, 1996; Vatn *et al.*, 2000; Edwards *et al.*, 2008; Lam-Himlin *et al.*, 2011; de Meij *et al.*, 2017). In this sense, concerning *C. ventriculi* detection, it is important to highlight two facts. Firstly, the diagnosis of such an agent must be associated with pathological findings, as its finding by chance does not point toward a clinical risk to the animal. Secondly, the smears evaluation in combination with histopathology of the abomasum is a strongly suggestive diagnostic method, as carried out in this study. Morphologically, the main differential diagnosis in this case is the presence of bacteria of the *Micrococcus* genus; these are smaller (0.5 μ m) and tend to form tightly packed clusters, a feature not seen with *C. ventriculi* (Lam-Himlin *et al.*, 2011).

Concerning the presence of *S. cerevisiae* in the milk replacer used to feed the kid goats, *S. cerevisiae* fermentation products have been extensively used in the dairy industry with beneficial effects on production parameters (Poppy *et al.*, 2012), modulation of the immune system (Zaworski *et al.*, 2014), improvement in rumen fermentation, stability of the gut microbiota (Brewer *et al.*, 2014), and reduction of diarrhea in calves (Alugongo *et al.*, 2017). 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 the *S. cerevisiae* within the kids' abomasum. They also ferment sugars, releasing ethyl alcohol and carbon dioxide similar to *C. ventriculi*. Corroborating this line of thought, there was a lot of yeast in the cytological smears of the kids affected by abomasal bloat. Therefore, more 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, where high infection rates were related to microbiota imbalances and poor hygiene. All environment/food samples (100.0%; 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. The large volume of milk ingested in a short time, high osmolarity, and the high temperature of the milk replacer associated with kid management are other strong reasons already verified in previous reports (DeBey et al., 1996; Van Kruiningen et al., 2009; Burgstaller et al., 2017).

Other important management findings highlighted in this study is the offering of *ad libitum* concentrate to the kids and their behavior to look for such food immediately after milking management. This can have favored the development of an environment with a large amount of milk and soluble carbohydrates contained within the abomasum. As already known, a high protein and carbohydrate concentration in the gut triggers the growth of *C. perfringens*, increasing the risk of enterotoxemia (Riddell and Kong, 1992; Roberfroid et al., 2010). Quinnet *et al.* (2011) also described various predisposing factors for the occurrence of enterotoxemia, like changes in diet, low proteolytic efficiency in neonates, trypsin inhibitors in colostrum, malnutrition, inefficient pancreatic secretion, no established intestinal flora, carbohydrates engorgement, and intestinal hypomotility (Sato et al., 1978).

Few reports on treatments of abomasal bloat have been described. However, most therapies included antibiotics (primarily penicillin and ampicillin), rumen “tonics” (including a wide variety of medicaments), non-steroidal anti-inflammatories (primarily flunixin meglumine), relieving the distension via a tube or trocar, Clostridial antitoxin, and fluid therapy as reviewed by Shoemaker *et al.* (2008). In the authors’ experience, the earlier the clinical signs (abomasal bloat) are identified, the better the animal’s prognosis.

Lastly, 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. Reversed genetics have demonstrated that alpha toxin is the main virulence factor for *C. perfringens* type A causing gas gangrene, and that vaccination with alpha toxoid could protect animals against this agent (Awad et al., 1995). Unfortunately, only a few vaccines against clostridial diseases for ruminants contain *C. perfringens* alpha toxoid in Brazil (Pires et al., 2017). 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 kid goats could be receiving low quality colostrum and immunization was not occurring efficiently. In this sense, the importance of quality plans for colostrum management in dairy farms is also emphasized.

In conclusion, an outbreak of abomasal bloat affected a dairy goat farm throughout the kidding season. The disease affected suckling kid goats among one and three months old with an acute clinical course of 6–12 hours until death. There was a mortality rate of 30% (66/221), and the agents *C. perfringens* type A and *C. ventriculi* were identified as the main ones involved with possible participation of *S. cerevisiae*. Among the possible associated factors, the erroneous use of milk replacer associated with errors in kid management favored decreases in abomasal emptying with local dysbiosis and clinical signs. Among the prophylactic measures, hygiene care, proper use of milk replacer, vaccination plan containing *C. perfringens* alpha toxoid associated with a good colostrum management were suggested.

ACKNOWLEDGMENTS

The authors thank the Universidade Federal Fluminense and Carlos Chagas Filho Foundation for Research Support in the State of Rio de Janeiro (FAPERJ). We also thank the dairy goat farm for their support throughout the study.

CONFLICTS OF INTEREST STATEMENT

The authors declare that no competing financial interests and that no conflicts of interest exist.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL

All clinical examinations were reviewed and approved by the Animal Care Committee of the Universidade Federal Fluminense (CEUA Protocol 855/16).

REFERENCES

- Al Rasheed, M.R.H., and C.G. Senseng, 2016: Sarcina ventriculi: Review of the literature. *Archives of pathology & laboratory medicine* **140** , 1441–1445. <https://doi.org/10.5858/arpa.2016-0028-RS>
- Alugongo, G.M., J.X. Xiao, Y.H. Chung, S.Z. Dong, S.L. Li, I. Yoon, Z.H. Wu, and Z.J. Cao, 2017: Effects of Saccharomyces cerevisiae fermentation products on dairy calves: Performance and health. *Journal of dairy science* **100** , 1189–1199. <https://doi.org/10.3168/jds.2016-11399>.
- Awad, M.M., A.E. Bryant, D.L. Stevens, and J.I. Rood, 1995: Virulence studies on chromosomal α -toxin and Θ -toxin mutants constructed by allelic exchange provide genetic evidence for the essential role of α -toxin in Clostridium perfringens-mediated gas gangrene. *Molecular microbiology* **15** , 191–202. <https://doi.org/10.1111/j.1365-2958.1995.tb02234.x>
- Brewer, M.T., K.L. Anderson, I. Yoon, M.F. Scott, and S.A. Carlson, 2014: Amelioration of salmonellosis in pre-weaned dairy calves fed Saccharomyces cerevisiae fermentation products in feed and milk replacer. *Veterinary microbiology* **172** , 248–255. <https://doi.org/10.1016/j.vetmic.2014.05.026>.
- Burgstaller, J., T. Wittek, and G.W. Smith, 2017: Invited review: Abomasal emptying in calves and its potential influence on gastrointestinal disease. *Journal of Dairy Science* **100** , 17–35, <https://doi.org/10.3168/jds.2016-10949>.
- Canale-Parola, E., 1970: Biology of the sugar-fermenting Sarcinae. *Bacteriological reviews* **34** , 82.
- Crowther, J.S., 1971: Sarcina ventriculi in human faeces. *Journal of medical microbiology* **4** , 343–350. <https://doi.org/10.1099/00222615-4-3-343>.
- de Meij, T.G., M.P. van Wijk, A. Mookhoek, and A.E. Budding, 2017: Ulcerative Gastritis and esophagitis in two Children with Sarcina ventriculi Infection. *Frontiers in medicine* **4** , 145. <https://doi.org/10.3389/fmed.2017.00145>.
- DeBey, B.M., P.C. Blanchard, and P.T. Durfee, 1996: Abomasal bloat associated with Sarcina-like bacteria in goat kids. *Journal of the American Veterinary Medical Association* **209** , 1468–1469.
- Edwards, G.T., N.G.A. Woodger, A.M. Barlow, S.J. Bell, D.G. Harwood, A. Otter, and A.R. Wight, 2008: Sarcina-like bacteria associated with bloat in young lambs and calves. *Veterinary Record* **163** , 391–393. <https://doi.org/10.1136/vr.163.13.391>.
- Freedman, J.C., J.R. Theoret, J.A. Wisniewski, F.A. Uzal, J.I. Rood, and B.A. McClane, 2015: Clostridium perfringens type A–E toxin plasmids. *Research in microbiology* **166** , 264–279. <https://doi.org/10.1016/j.resmic.2014.09.004>.
- Goodsir, J., and G. Wilson, 1842: History of a case in which a fluid periodically ejected from the stomach contained vegetable organisms of an undescribed form. *Edinburgh Medical and Surgical Journal* **57** , 430.
- Kamatchi, V., N. Babu, S. Sankari, and E. Rajesh, 2015: Imprint cytology. *Journal of Pharmacy and Bioallied Sciences* **7** , 207. <https://doi.org/10.4103/0975-7406.155905>.
- Khan, M.A., A.Z. Durrani, S.B. Khan, N.U. Khan, M.A. Khan, K. Prince, M. Ali, G. Rashid, and A.U. Khan, 2020: Biomarkers for Pathogenic Clostridium perfringens in Small Ruminants of Khyber Pakhtunkhwa,

Pakistan. *Pakistan Journal of Zoology* **52** . <https://doi.org/10.17582/journal.pjz/2020.52.1.107.114>.

Kiernan, J.A., 2008: Histological and histochemical methods: theory and practice. *Bloxham, UK: Scion* .

Konishi, M., Y. Nagura, N. Takei, M. Fujita, K. Hayashi, M. Tsukioka, T. Yamamoto, K. Kameyama, H. Sentsui, and K. Murakami, 2011: Combined eradication strategy for CAE in a dairy goat farm in Japan. *Small ruminant research* **99** , 65–71. <https://doi.org/10.1016/j.smallrumres.2011.03.051>.

Lam-Himlin, D., A.C. Tsiatis, E. Montgomery, R.K. Pai, J.A. Brown, M. Razavi, L. Lamps, J.R. Eshleman, B. Bhagavan, and R.A. Anders, 2011: Sarcina organisms in the gastrointestinal tract: a clinicopathologic and molecular study. *The American journal of surgical pathology* **35** , 1700. <https://doi.org/10.1097/PAS.0b013e31822911e6>.

Leite Filho, R.V., M.V. Bianchi, G. Fredo, E.C. de Oliveira, C.J.M. Laise, D. Driemeier, and S.P. Pavarini, 2016: Emphysematous abomasitis in a lamb by bacteria of the Sarcina genus in Southern Brazil. *Ciência Rural* **46** , 300–303. <https://doi.org/10.1590/0103-8478cr20151078>.

Meer, R.R., and J.G. Songer, 1997: Multiplex polymerase chain reaction assay for genotyping *Clostridium perfringens*. *American journal of veterinary research* **58** , 702–705.

Merck KGaA, 2014: Cytodiagnosis Staining Methods [Online] Available at <https://www.merckmillipore.com/BR/pt/search/Cytodiagnosis%20Staining%20Methods?search=&TrackingSearchType=SE+homepage-search-box+-+OLD&SearchContextPageletUUID=&SearchTerm=Cytodiagnosis+Staining+Methods&search=> (accessed November 14, 2019).

Miyashiro, S., A.F.C. Nassar, C. Del Fava, A.D. Cabral, and M. Silva, 2007: *Clostridium perfringens* types A and D associated with enterotoxemia in an 18-month-old goat. *Journal of Venomous Animals and Toxins including Tropical Diseases* **13** , 885–893. <https://doi.org/10.1590/S1678-91992007000400017>

Mowlem, A., 1984: Artificial rearing of kids. *Goat Veterinary Society* .

Panciera, R.J., M.J. Boileau, and D.L. Step, 2007: Tympany, acidosis, and mural emphysema of the stomach in calves: report of cases and experimental induction. *Journal of veterinary diagnostic investigation* **19** , 392–395. <https://doi.org/10.1177/104063870701900409>.

Peek, S.F., S.D. Semrad, and G.A. Perkins, 2003: Clostridial myonecrosis in horses (37 cases 1985–2000). *Equine veterinary journal* **35** , 86–92. <https://doi.org/10.2746/042516403775467513>.

Pires, P.S., R. Ecco, R.O.S. Silva, M.R. de Araújo, F.M. Salvarani, L.G.D. Heneine, C.A. de Oliveira Júnior, and F.C.F. Lobato, 2017: A retrospective study on the diagnosis of clostridial myonecrosis in ruminants in Brazil. *Ciência Rural* **47** . <https://doi.org/10.1590/0103-8478cr20160492>.

Poppy, G.D., A.R. Rabiee, I.J. Lean, W.K. Sanchez, K.L. Dorton, and P.S. Morley, 2012: A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of *Saccharomyces cerevisiae* on milk production of lactating dairy cows. *Journal of dairy science* **95** , 6027–6041. <https://doi.org/10.3168/jds.2012-5577>.

Quinn, P.J., B.K. Markey, F.C. Leonard, P. Hartigan, S. Fanning, and Es. Fitzpatrick, 2011: Veterinary Microbiology and Microbial Disease. John Wiley & Sons.

Riddell, C., and X.-M. Kong, 1992: The influence of diet on necrotic enteritis in broiler chickens. *Avian diseases* 499–503.

Ripoll, G., M.J. Alcalde, M.G. Córdoba, R. Casquete, A. Argüello, S. Ruiz-Moyano, and B. Panea, 2019: Influence of the Use of Milk Replacers and pH on the Texture Profiles of Raw and Cooked Meat of Suckling Kids. *Foods* **8** , 589. <https://doi.org/10.3390/foods8110589>.

Roberfroid, M., G.R. Gibson, L. Hoyles, A.L. McCartney, R. Rastall, I. Rowland, D. Wolvers, B. Watzl, H. Szajewska, and B. Stahl, 2010: Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*

104 , S1–S63. <https://doi.org/10.1017/S0007114510003363>.

Roeder, B.L., M.M. Chengappa, T.G. Nagaraja, T.B. Avery, and G.A. Kennedy, 1987: Isolation of *Clostridium perfringens* from neonatal calves with ruminal and abomasal tympany, abomasitis, and abomasal ulceration. *Journal of the American Veterinary Medical Association* **190** , 1550–1555.

Roeder, B.L., M.M. Chengappa, T.G. Nagaraja, T.B. Avery, and G.A. Kennedy, 1988: Experimental induction of abdominal tympany, abomasitis, and abomasal ulceration by intraruminal inoculation of *Clostridium perfringens* type A in neonatal calves. *American journal of veterinary research* **49** , 201–207.

Sato, H., Y. Yamakawa, A. Ito, and R. Murata, 1978: Effect of zinc and calcium ions on the production of alpha-toxin and proteases by *Clostridium perfringens*. *Infection and immunity* **20** , 325–333.

Shoemaker, D.E., L.T. Midla, and P.J. Rajala-Schultz, 2008: Factors Associated with Acute Bloat Syndrome in Pre-Weaned Dairy Heifers. pp. 85–90. In: *Proceedings of the 17th Annual Tri-State Dairy Nutrition Conference, Fort Wayne, Indiana, USA, 22-23 April, 2008* . Ohio State University.

Singh, K., 2019: Emphysematous gastritis associated with *Sarcina ventriculi*. *Case reports in gastroenterology* **13** , 207–213. <https://doi.org/10.1159/000499446>.

Songer, J.G., and D.W. Miskimins, 2005: Clostridial abomasitis in calves: Case report and review of the literature. *Anaerobe* **11** , 290–294. <https://doi.org/10.1016/j.anaerobe.2004.12.004>.

Stevenson, M., S. Firestone, and A. Wiethoelter, 2017: An Introduction to Veterinary Epidemiology. Australia: Faculty of Veterinary and Agricultural Sciences, University of Melbourne.

Tolentino, L.F., N. Kallichanda, B. Javier, R. Yoshimori, and S.W. French, 2003: A case report of gastric perforation and peritonitis associated with opportunistic infection by *Sarcina ventriculi*. *Laboratory Medicine* **34** , 535–537. <https://doi.org/10.1309/CDFF04HE9FHDQPAN>

Uzal, F.A., J.E. Vidal, B.A. McClane, and A.A. Gurjar, 2010: *Clostridium perfringens* toxins involved in mammalian veterinary diseases. *The open toxinology journal* **2** , 24.

Van Kruiningen, H.J., C.A. Nyaoke, I.F. Sidor, J.J. Fabis, L.S. Hinckley, and K.A. Lindell, 2009: Clostridial abomasal disease in Connecticut dairy calves. *The Canadian Veterinary Journal* **50** , 857.

Vatn, S., M.A. Tranulis, and M. Hofshagen, 2000: *Sarcina*-like bacteria, *Clostridium fallax* and *Clostridium sordellii* in lambs with abomasal bloat, haemorrhage and ulcers. *Journal of comparative pathology* **122** , 193–200. <https://doi.org/10.1053/jcpa.1999.0363>.

Yen, J.-H., H.S. Huang, C.J. Chuang, and S.-T. Huang, 2019: Activation of dynamin-related protein 1-dependent mitochondria fragmentation and suppression of osteosarcoma by cryptotanshinone. *Journal of Experimental & Clinical Cancer Research* **38** , 42. <https://doi.org/10.1186/s13046-018-1008-8>.

Zaworski, E.M., C.M. Shriver-Munsch, N.A. Fadden, W.K. Sanchez, I. Yoon, and G. Bobe, 2014: Effects of feeding various dosages of *Saccharomyces cerevisiae* fermentation product in transition dairy cows. *Journal of dairy science* **97** , 3081–3098. <https://doi.org/10.3168/jds.2013-7692>.

FIGURE CAPTIONS

FIGURE 1. A and B) Kid goat of Saanen breed (30 days old) showing a distended abdomen mainly in the right and left paralombar fossa; C) Pens with concrete floors covered with shavings. Lateral walls 1.5 meters high without windows and poor ventilation; D) Overcrowded solarium area.

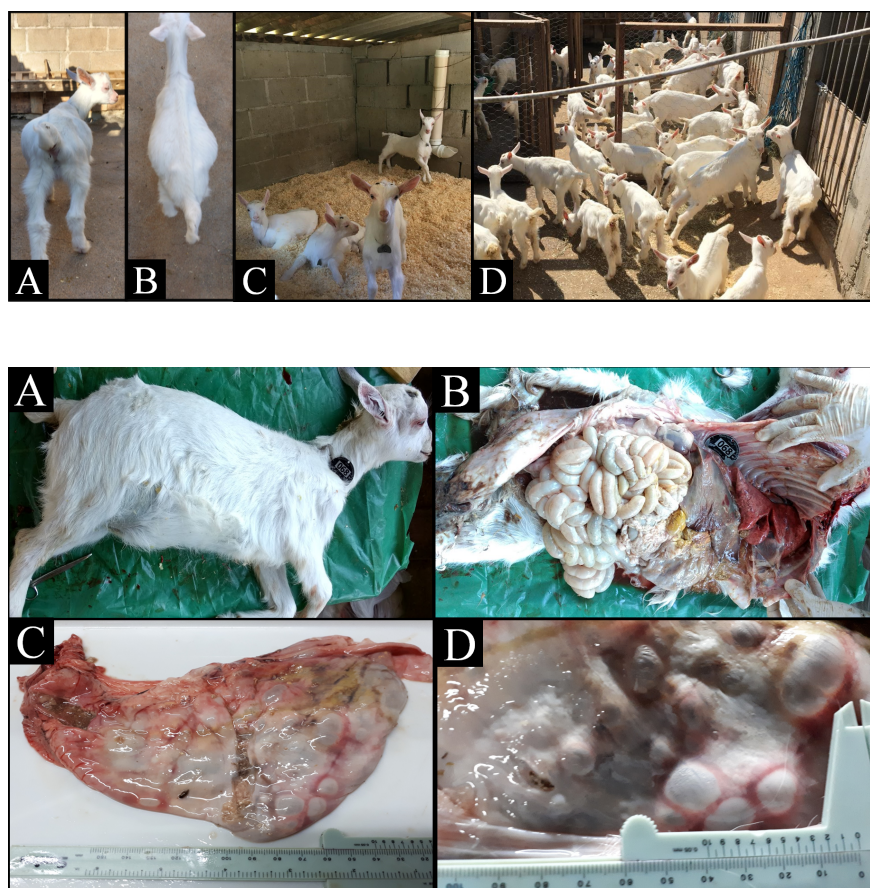
FIGURE 2. A) Kid goat of Saanen breed (45 days old; Kid C) found dead with intense abdominal bloating and subcutaneous emphysema; B) Advanced state of autolysis, free abomasal contents in the abdominal cavity and peritonitis; C) Abomasal congestion and diffuse emphysema in the abomasal wall; D) Details of the emphysema in the abomasal wall.

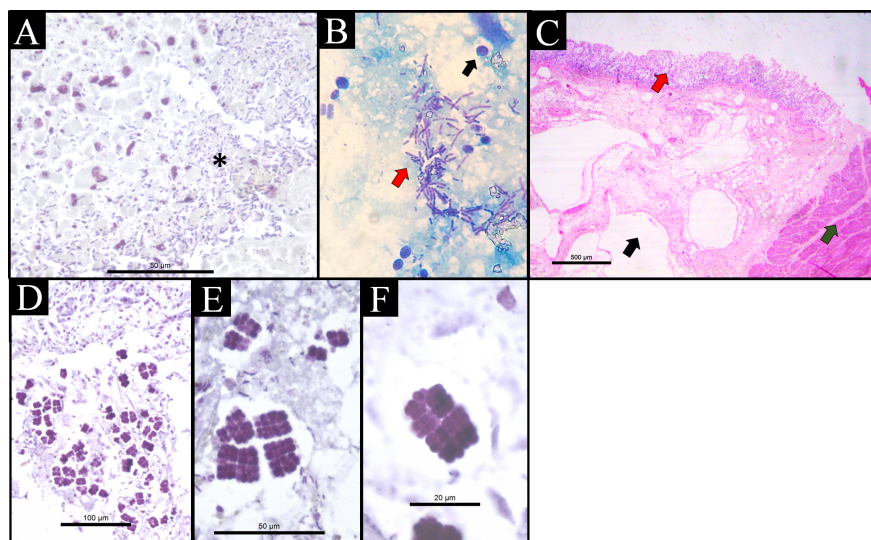
FIGURE 3. Photomicrograph of abomasal tissue. A) Hyalinization of the abomasal wall with *C. perfringens* characterized as basophilic rods (Gram+; *). Histopathology staining by GRAM. Magnification of 630×. B) Imprinting of abomasal mucosa - *C. perfringens* characterized as basophilic rods (black arrow) and round to ovoid yeasts compatible with *Saccharomyces cerevisiae* (red arrow; 5–10 µm). Rapid Panoptic Staining. Amplification of 630X. C) Abomasal tissue. Emphysematous abomasitis – gas accumulated at the submucosa of the abomasum (black arrow). Mucosa (red arrow). Muscle tunics (green arrow). HE staining. Magnification of 40×. D, E and F) *C. ventriculi* in the abomasal mucosa. Histopathology staining by GRAM. Magnification of 200×, 630×, and 1000×, respectively.

LIST OF TABLES

TABLE 1. Possible associated factors found in the management of lactating kid goats in the dairy goat farm along with suggestions made for correction and prevention.

TABLE 2. Overall results obtained from pathology (macroscopy and microscopy), molecular biology, bacteriology, and cytology data obtained from the outbreak of emphysematous abomasitis in suckling kid Saanen goats raised under intensive management.





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