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CASE REPORT

Clinico-Morphological Studies in a Clinical Case of Emphysematous Abomasitis in a Lamb Herd in Bulgaria

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Abstract

The present report describes a clinical case of emphysematous abomasitis in lambs caused by Sarcina spp. The lambs were aged from the 24th hour after birth to the 35th day additionally supplemented with milk replacer due to the reduced amount of milk in the mothers, and from the second week they began to accept "ad libitum" starter granulated feed and ground corn. After which they began to show the following signs: severely swollen abdomen and difficult breathing, cachexia, lying down, lethargy, pale conjunctivae, feces were pale yellow mixed with mucus, internal body temperature was normal. In a period of 10 days, 50 lambs became ill and 10 died. At the performed autopsy, the abomasum was filled with gases, its contents had a whitish color and mucous consistency. The mucosa and submucosa were diffusely hyperemic, and had an edematous and emphysematous appearance. On the surface of the mucosa, desquamated epithelial cells mixed with exudate containing fibrin threads, numerous degenerated neutrophils, eosinophils, macrophages and grouped bacteria of the genus Sarcina spp. were observed. The combined use of pathoanatomical and histopathological examination are effective and reliable methods for diagnosis and differential diagnosis of emphysematous abomasitis in lactating lambs caused by bacteria of the genus Sarcina. Characteristic morphologies of bacteria allows us to distinguish them histologically from clostridia, as well as from other etiological agents

Keywords: Emphysematous abomasitis, lambs, pathology, Sarcina spp.

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INTRODUCTION

Abomasal diseases are relatively common and this may be partially attributed to feeding practices and dietary management: disruption in nursing patterns, large amounts of milk at infrequent feedings, poor milk hygiene, and milk replacers with high concentrations of carbohydrate or protein have all been associated with abomasal bloat. Decreased gastrointestinal motility also promotes abomasal bacterial overgrowth and can occur for a number of reasons. Numerous reports show an association between ulcerative, emphysematous, or necrotic abomasitis and the presence of Clostridium spp. and Sarcina-like bacteria in small ruminants (Simpson et al 2018, Collins-Webb et al 2021). Sarcina is a gram-positive, anaerobic, spore-forming coccus first identified by Goodsir in a patient with gastric fermentation in 1842, then by Dr Ferrier in the blood of patients in 1872, who noted its association with delayed gastric emptying (Sopha et al 2015). He found that this organism was 1.8-3 mm in diameter, spherical in shape, grouped in bundles of 4, 6, 8 and more bacteria,

resistant to acidic pH. This characteristic morphological configuration is the result of cell division in two growth planes of the microorganism (Bhagat 2015). Abomasal bloat and emphysematous abomasitis associated with the Sarcina genus of bacteria is a rare disease reported in young ruminants such as calves, lambs and kids. Pathogenesis of abomasal bloat is not yet fully elucidated. (Leite Filho et al 2016).

The pathogenetic mechanism of gas accumulation in the abomasum, edema and emphysematous changes on the mucosa and submucosa are still not fully understood. Western authors believe that this syndrome has a multifactorial origin such as: the excessive fermentation of carbohydrates in the abomasum, due to the presence of gas-forming bacteria (*Sarcina* spp., *Lactobacillus* spp. and *Clostridium* spp.) in its contents. Excessive intake of foods rich in carbohydrates and proteins, unbalanced rations, dysbacteriosis and others (Panciera et al 2007).

Debey et al (1996) described a clinical case of emphysematous abomasitis in 200 goat kids at 25



days of age with a 70% mortality rate. Authors such as (Lewis and Naylor 1998) report that abomasal erosions, ulcers and emphysematous changes of the abomasum in lambs usually occur between three and ten weeks of age, secondary to maldigestion caused by feeding errors. Similar macroscopic lesions of emphysematous abomasitis and dilatation of the abomasum were described (Doll 1991) in lactating calves. Some authors histologically be classified ulcers if the necrosis of the abomasal wall reaches deeper than the lamina muscularis mucosae into the submucosal 4 types (Hund and Wittek 2018).

Authors such as Viana Leite et al (2016) have implicated bacteria of the genus Sarcina as the main etiological agent of emphysematous abomasitis in young ruminants. They describe the disease in 80 lambs at the age of thirty days. According to them, the disease appeared a few days after the introduction of concentrated feed to the diet. At autopsy, the following pathological changes were observed: the abomasum was distended by gases with diffusely thickened walls and emphysema. Microscopically, they found diffuse emphysema and multifocal necrosis in the abomasum, as well as coccishaped microorganisms 2 mm in diameter, arranged in tetrahedral shapes resembling bacteria of the genus Sarcina. The results of the epidemiological, clinical, pathoanatomical and histopathological studies were reported in detail. A diagnosis of emphysematous abomasitis in lambs caused by bacteria belonging to the genus Sarcina was then made.

After the literature review and becoming familiar with the multifactorial etiology of diseases of the abomasum, as part of the gastrointestinal pathology in adolescent ruminants. We present a clinical case of emphysematous abomasitis in a lamb caused by *Sarcina* spp. describing the observed macroscopic and microscopic changes in the digestive system in lambs with the aim of their application in the pathomorphological diagnosis of gastrointestinal diseases.

CASE PRESENTATION

Farmer-owner consent was obtained for the procedures undertaken and the use of the data for research purposes, his study was approved.

The presented report describes a clinical case of emphysematous abomasitis in lambs, which occurred with 70% morbidity and 20% mortality, with significant retardation in growth and development of all lambs on a private sheep farm in Southern Bulgaria. The following diagnostic studies were performed: pathoanatomical, antigenic, histopathological and microbiological. The herd was of the Romanov sheep breed consisting of 200 ewes and 70 newborn and adolescent lambs aged from the 24th hour after birth to the 35th day. The sheep were dewormed but not vaccinated against contagious diseases during pregnancy. The animals were raised in poor zoohygienic conditions, as the approximate daily ration of one sheep was 500 g of concentrated feed and meadow hay "ad libitum". All lambs were vaccinated on the first day after birth against clostridial infections, but not against other diseases. The lambs were additionally supplemented with milk replacer due to the reduced amount of milk in the mothers, and from the second week they began to freely accept starter granulated feed and ground corn, as they were significantly behind in growth and development. After a few days, they began to show the following signs: a very swollen abdomen and difficulty breathing, cachexia, colic cramps, lying down, lethargy, pale conjunctivae, feces were pale yellow mixed with mucus, internal body temperature was normal. After several days of illness, the animals died, the cachectic and retarded lambs were discarded by the farmer. The patients were treated, by probing and oral administration of gentamicin (2-4 mg/kg) and intramuscularly administered penicillin antibiotic (1ml/10kg), busculizin (hyoscine butylbromide, 0,2ml/1kg) and analgin (metamizole sodium 300 mg/ml, 2,5 gr/100kg) but without success. Despite treatment over a period of 10 days, 50 lambs became ill and 10 died. Four carcasses of lambs aged between 20 and 30 days, weighing 6-8 kg, were provided for necropsy and subsequent diagnostic studies by the farm owner.

Histopathology and bacteriological studies

Rapid antigen diagnostic tests for current enteropathogens causing neonatal diarrhea in ruminants were performed (5 valent antigen tests, Rainbow calf scour 5 BIO K 306 Detection of Rotavirus, Coronavirus, *E. coli* F5, Cryptosporidium parvum (BIOX Diagnostics, Belgium). After in which tissue samples for histopathological examination measuring 1 cm x 1 cm were obtained from abomasum, duodenum, jejunum with mesenteric lymph nodes, ileum, cecum, colon and rectum. Materials for histopathological examination were fixed in 10% neutral buffered formalin for 48–72 h and embedded in paraffin. Sections with a thickness of 4 μ m were prepared from the obtained paraffin blocks using a "Leica" RM 2235 microtome and stained conventionally with hematoxylineosin (H/E).

Also, samples from the parenchymal organs (lung, liver, spleen and kidney), blood from the heart and a ligated section of the small intestine were used for bacteriological studies. Cultures were prepared on blood agar with 5% sheep erythrocytes, McConkey agar, trypticase-soy broth, selenite broth. All these cultures were incubated at 37°C for 24 hours under aerobic conditions. For the isolation of

anaerobic microorganisms, Zeisler's blood sugar agar and thioglycolate medium (fluid thioglycolate medium; Difco Laboratories) were used, and cultures were cultivated for 24-48 hours at 37°C and anaerobic conditions.

Results and discussion

Macroscopic findings

In the pathological examination performed according to the standard autopsy protocol of 4 lambs carcasses, the following macroscopic changes were found: undersized carcass, cachexia, pale conjunctival mucous membranes, bilateral mucous discharge from the nasal cavity and medial eye angle, yellowish staining in the perianal area and pelvic limbs, highly swollen carcass and enlarged abdomen. In all four lambs carcasses, the abomasum occupied one third of the volume of the abdominal cavity. It was greatly enlarged, as a result of its gas filling, crepitation was detected upon palpation. On dissection, its contents were whitish in color and slimy in consistency. The mucosa and submucosa were diffusely hyperemic, with an edematous and emphysematous appearance of the folds (Figure 1). Numerous punctate hemorrhages and erosions measuring 1 mm were observed in places. A transverse section of the abomasum wall between the mucosa and submucosa, as well as in the abomasal folds, revealed numerous gas bubbles with a diameter of 1 mm to 1.5 centimeters (Figure 2). The rest of the foregut was filled with undigested roughage and concentrated feed. Specialists in the field such as Estela et al (2024) describe macroscopic changes in a case of fibrinonecrotizing abomasitis in lambs, with the following changes: highly thickened abomasum walls, transmural edema, dark red mucosa, multiple subserosal hemorrhages, and mucosal ulceration in the pyloric and fundus regions of the abomasum, fibrinous peritonitis and pleuropericarditis. A 1.5 cm diameter mucosal abscess was observed in one lamb. Balaro et al (2022) found macro changes similar to ours in goat kids with clostridial abomasitis: dehydration, pale visible mucous membranes, ascites, flatulence, congestion of the great vessels, emphysematous abomasitis, blood clots in the left ventricle, renal congestion and pulmonary consolidation of the cranial lobes. Author collective Marcelino et al (2021), report a case of emphysematous gastritis in a man caused by sarcinia. The gastric mucosa was congested, edematous and hemorrhagic imbibed, a 6 cm perforation was observed in the region of the antrum of the stomach.

The serosa of the small intestine was hyperemic and thinned with visualization of the intestinal contents. The intestinal mucosa was hyperemic and edematous, and the contents had a mucous consistency and a yellow-green color. The colon was severely distended, and the mesenteric lymph nodes were intensely red and



folds of the abomasum are ballooned and filled with gas (arrows), lamb

enlarged. Ecchymotic hemorrhages were observed on the peritoneum. The liver and kidneys were congested. The bladder was enlarged and overflowing with urine. A section of the lung revealed the presence of foamy fluid in the trachea and bronchi, palpation revealed compacted areas with a doughy consistency around the bifurcation of the trachea. Bilaterally, on the surface and periphery of the lung, pale foci crepitating on palpation were found.

Microscopic findings

From the performed routine histopathological examinations in the abomasum, the following microlesional manifestations were established: the blood vessels in the submucosa were highly dilated and overflowing with erythrocytes, a result of the hyperemia that occurred. Transmural hemorrhages were also present in places. Diffuse emphysema was found affecting the mucosa, submucosa, musculature and serosa of the



Figure 2. Cross-sections of different sections of the abomasum, strongly thickened mucosa and submucosa due to accumulation of gases - emphysema (arrows), lamb

abomasum (Figure 3). There were also focal areas of hyalinization in the muscle layer. Researchers such as Balaro et al (2022) reported a clinical case of clostridial abomasitis in a goat kid with hyalinization of the muscle layer of the abomasum. Other authors (Collins-Webb et al 2021) reported necroulcerative reticulorumenitis in lambs with coagulation necrosis of the forestomach mucosa and emphysema of equally necrotic submucosa. On the surface of the mucosa, in addition to degenerative necrobiotic changes and desquamated epithelial cells, an exudate composed of fibrin threads mixed with numerous degenerated neutrophils, eosinophils, macrophages and basophilic coccoid bacteria, grouped in cube-shaped packages 2x2 and more in number, was observed. With a characteristic morphology of microorganisms of the genus Sarcina spp. (Figure 4). Authors such as Sopha et al (2015) and Bhagat (2015) in previous studies in animals and humans the histology and cytology methods have been often used because bacteria of Sarcina genus have an unique morphological feature that is not found in other agents, as well as its extremely difficult isolation of which in most cases revealed negative results. The fibrinous exudate mixed with neutrophils and gas bubbles penetrated deeply into the mucosa, submucosa and muscle layer of the organ. Between the glands there was a highly expressed diffuse edema, hyperplasia and hypersecretion, and in their epithelium, degenerative and necrobiotic processes were strongly expressed (Figure 5). These results of ours coincide and overlap to some extent with those described by Viana Leite et al (2016), in outbreaks of the disease in lambs in the territory of Brazil. Melendez and Poock (2018) found similar microscopic changes in the abomasum of lactating calves with clostridial abomasitis. Prescott et al (2016) reported that emphysematous changes in the abomasum were frequently observed, both in brazot and in other clostridiosis in lambs, and were rarely found in fibrinous abomasitis caused by Manhaemia haemolytica.



In fibrinonecrotizing abomasitis in lambs, Estela et al (2024) described microscopically the presence of coagulative necrosis in the mucosa. Bacterial colonies around the wall of medium and large veins and venules and emphysema in the submucosa, emboli and fibrin thrombi in the vessels mixed with neutrophils and bacteria. In the other examined segments of the gastrointestinal tract (duodenum, jejunum, ileum, cecum, colon and rectum), only catarrhal desquamative processes affecting the surface part of the villi and congestion of the vessels in the submucosa were observed. In the mesenteric lymph nodes, the blood vessels were hyperemic and in the medulla/core, there was edema.

The obtained results of the performed antigen tests were negative for *Cryptosporidium parvum*, rotaviruses, coronaviruses and *Escherichia coli*. No pathogenic microorganisms were found and from the conducted microbiological study for the isolation and identification of aerobic and anaerobic microbial agents.



Figure 3. Diffuse emphysema (arrows) affecting the mucosa, submucosa and muscle layer of the abomasum, lamb, H/E, bar=10 μm



Figure 5. Multifocal edema (arrows), hyperplasia and hypersecretion of the glands, with pronounced degenerative necrobiotic processes in their epithelium, abomasum, lamb, H/E, bar=10 μm

Conclusion

In conclusion, we believe that anamnesis data together with clinical and epidemiological studies are essential for elucidating the predisposing factors and their interrelationship between etiology, pathogenesis and clinical manifestation in diseases of the abomasum. The combined use of pathoanatomical and histopathological examination are effective and reliable methods for diagnosis and differential diagnosis of emphysematous abomasitis in lactating lambs caused by bacteria of the genus Sarcina. Their characteristic morphology allows us to distinguish them histologically from clostridia, as well as from other etiological agents causing abomasitis in adolescent ruminants.

DECLARATIONS

Competing Interests

Authors declare that there are no conflicts of interest related to the publication of this article.

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Availability of Data and Materials

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

Ethical Statement

Informed consent was obtained from the animal owner. Owners' consent was obtained for the procedures undertaken and the use of the data for research purposes.

Author Contributions

Motivation / Concept: IK; Design: IK; Control/Supervision: IK Data Collection and Processing: IK; Analysis and Interpretation: IK; Literature Review: IK; Writing the Article: IK; Critical Review: IK.

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