

Diagnosis of *Sarcina ventriculi*-derived haemorrhagic abomasitis in lambs by histopathology and real-time PCR

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Received February 24, 2022

Accepted June 14, 2022

Abstract

This study aimed to diagnose *Sarcina ventriculi* in lambs with haemorrhagic abomasitis using histopathological and real-time PCR methods. The material used in this study consisted of 43 abomasum tissues recovered from lambs presenting bleeding, ulcer, gas or a combination of these in the abomasum, that were brought for necropsy to the Department of Pathology of the Veterinary Faculty, Selcuk University. The recovered samples were stored in a 10% formaldehyde solution for histopathological examinations and in Eppendorf tubes at –20 °C for PCR examinations. All the samples were analyzed by histopathological and PCR methods. While *S. ventriculi* pyruvate decarboxylase (PDC) amplicon was determined by real-time PCR in 17 cases, the packaged form of *Sarcina*-like bacteria was found microscopically in 3 cases only. In this study, the diagnosis of *S. ventriculi* in cases of lambs presenting bleeding, ulcers and gas in the abomasum or haemorrhagic abomasitis simultaneously at the necropsy was performed using histopathological and real-time PCR methods; in parallel, the real-time PCR method for the diagnosis of *S. ventriculi*-derived haemorrhagic abomasitis in lambs was also optimized.

Necropsy, abomasitis, Sarcina-like bacteria, pyruvate decarboxylase, real-time PCR

Abomasitis is defined as the inflammation of ruminant stomachs. In lambs, abomasitis usually progresses as gastroenteritis with enteritis. According to the pathological anatomical features, the abomasitis inflammation may present in four different forms: catarrhal, fibrinous, diphtheroid-necrotic and haemorrhagic abomasitis. Haemorrhagic abomasitis is known as the most common lesion in the abomasum caused by infectious agents and is usually associated with clostridial infections (Lewis 2007; Benavides et al. 2015; Çiftçi et al. 2015). Haemorrhagic abomasitis is mostly encountered in young ruminants. *Sarcina* species, as well as other clostridial species, are of great importance in its aetiology. Agents such as *C. septicum*, *C. perfringens* type A, *C. sordellii* and *Sarcina* spp. alone or combined have been reported as the most common causes of haemorrhagic abomasitis. *Sarcina* spp. and *Clostridium* spp. are also recognized as important gas producers (Prescott et al. 2016; Vatn et al. 2000).

Sarcina species was first reported by John Goodsir in 1842 in a patient suffering from stomach pain, gas, and vomit (Edwards et al. 2008). *Sarcina* species are gram-positive, cocciform bacteria of the *Clostridiaceae* family with a diameter of 1.8–3.0 µm and observed in packages of 8 or more spherical-shaped bacteria (Canale-Parola and Wolfe 1960; Canale-Parola 1986). It has been reported in different studies that *Sarcina* species is an aetiological agent in the abomasal swelling and death of farm animals (DeBey et al. 1996; Schemm et al. 1999; Vatn et al. 2000; Aubry 2004; Dillard and Anttila 2005). In cases of *Sarcina* species-related abomasitis, *Sarcina*-like microorganisms can be observed in histological smears (Edwards et al. 2008; Van Kruiningen et al. 2009). In addition, it has been reported that in lambs with abomasal swelling, it can be seen

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microscopically on the mucosal surface of the abomasum and surrounded by neutrophil granulocytes in the mucus over the mucosal ulcers (Vatn et al. 2000; Prescott et al. 2016).

Vatn et al. (2000) reported that in a study performed on 2–5 week old lambs, *Sarcina*-like bacteria was detected in 79% of the lambs in which gas accumulation, bleeding, and ulcer combinations were observed in the abomasum. They also reported that *Sarcina*-like bacteria was seen in 94% (44/47) of the lambs with abomasal gas, whilst only in the 45% (9/20) of the lambs with ulcers and/or bleeding, or both. The same researchers also reported that bezoars were frequently found in lambs with *Sarcina*-like bacteria. DeBey et al. (1996), on the other hand, investigated the presence of *Sarcina* species in kids with a history of 6–10 weeks of abdominal swelling, finding that of 10 deceased kids, abdominal gas was present in 7 and, histologically, *Sarcina*-like bacteria were found in 9 of them.

In various studies, two *Sarcina* species, *S. ventriculi* and *S. maxima*, were reported, and it was stated that these species are anaerobic, able to ferment sugars, and are common in environmental areas such as cereal grains and soil (Canale-Parola 1970; Canale-Parola 1986). These two species survive at very low pH and are able to cause rapid swelling and abomasal rupture with the production of carbon dioxide as a result of fermentation (Edwards et al. 2008). In a study, only 1 of 12 cases in which *Sarcina*-like bacteria were seen in smears showed growth in culture, indicating that it is difficult to determine the agent by culture after death (Vatn et al. 2000).

Since it is difficult to reproduce *Sarcina* species in culture which is thought to play an important role in the aetiology of abomasitis cases, this study was aimed at diagnosing *S. ventriculi* in lambs with haemorrhagic abomasitis using the real-time PCR method and the histopathological findings.

Materials and Methods

The material for the study consisted of 43 abomasum tissues retrieved from the lambs brought for necropsy to Pathology Department of the Veterinary Faculty of the Selcuk University in 2021, and presenting bleeding, ulcer gas or a combination of these at the necropsy. These 43 samples consisted of abomasum samples which were negative as a result of immunohistochemical staining for *Clostridium* spp., which is routinely applied to haemorrhagic abomasitis cases in our laboratory. The samples were stored in a 10% formaldehyde solution for the histopathological examinations and at –20 °C in an Eppendorf tube for the PCR examinations. As a control, 6 healthy abomasum tissues were collected from a commercial slaughterhouse and stored similarly. The study's compliance with the ethical principles was approved by SÜVDAMEK (No. 2021/69).

Histopathological examination

After the tissue samples collected for histopathological examination were fixed in a 10% formaldehyde solution for one day, routine tissue follow-up procedures were applied. Sections of 5 µm thickness were excised from the obtained paraffin blocks and stained with haematoxylin and eosin (Luna 1968). Preparations were examined under a light microscope (Olympus BX51, Tokyo, Japan) and photographed (Olympus EP50).

Immunohistochemical staining

Sections were taken from paraffin blocks on 4–5 µm adhesive slides. The sections were kept in an oven at 60 °C for 20 min and then paraffin extraction and rehydration processes were performed on the sections. After washing the sections with PBS, endogenous peroxidase inactivation was achieved by keeping them in 3% H₂O₂ for 10 min. To reveal the antigen in the tissues, they were treated with antigen retrieval solution (citrate buffer pH 6.0) for 2 × 5 min at 500 Watts. Afterwards, sections washed 2 × 5 with PBS, IHC staining were performed with Ultra Vision Detection System Anti-Polyvalent, HRP (Ready-To-Use, TP-060-HL, Lab Vision, USA) immunohistochemistry kit in accordance with the manufacturer's recommendations. Relevant primers were obtained from the Istanbul Pendik Veterinary Research Institute. Then, 3,3 diaminobenzidine was used as chromogen and counterstained with Mayer's Haematoxylin Solution. In the last stage, it was passed through the alcohol and xylene series twice, and covered with lamellas.

Real-time PCR analysis

The analysis was performed with the Light Cycler 2.0 real-time PCR device using the primer probes synthesized by a private company for *S. ventriculi* (Table 1). Primers were designed using sequence data from the GenBank to target the terminal region of the 16S rRNA gene (AF110272.1) and the pyruvate decarboxylase (PDC) gene (AF354297.1) of *S. ventriculi*. Deionized water was used as the negative control. The DNA isolation from the

tissue samples taken for PCR examinations was performed using a commercial DNA isolation kit (Roche, MagNA Pure LC DNA Isolation Kit, Cat No; 03264785001) in the sequence indicated. Two hundred µl of homogenized tissue, 200 µl of tissue lysis binding buffer, 40 µl of proteinase K were placed in an Eppendorf tube and vortexed at 55 °C for 1 h. Five µl of lysozyme were added and incubated at 37 °C for 15 min. Then, 200 µl of binding buffer were added and incubated at 70 °C for further 10 min. After 10 min of incubation, 100 µl of isopropanol were added and mixed. The mixture was transferred to filter tubes and centrifuged at 8,000 g for 1 min. The lower tube was discarded and replaced with a clean tube. Five hundred µl of inhibitor removal buffer were placed in the filter tube and centrifuged at 8,000 g for 1 min. The lower tube was discarded and replaced with a clean tube. Five hundred µl of wash buffer were added to the filter tube and centrifuged at 8,000 g for 1 min. The lower tube was discarded and replaced with a clean tube. This process was repeated twice. The liquid in the lower tube was discarded, and the tube was placed under the filter tube again, then the tube was centrifuged at 13,000 g for 10 min. Filtered tubes were transferred to 1.5 ml DNase, RNase free Eppendorf tubes. Two hundred µl of elution buffer heated at +70 °C were added and then centrifuged at 8,000 g for 1 min. The obtained products were stored at -20 °C until loading into the PCR device. Reaction mixture; a total of 15 µl were prepared by the addition of nuclease-free water 3.8 µl, forward primer (20uM) 0.5 µl, reverse primer (20uM) 0.5 µl, TaqMan probe (20uM) 0.2 µl, enzyme and dNTP mix 10 µl. The mixture was spun and transferred from the reaction mixture to the capillaries at a rate of 15 µl for each reaction. Five µl of sample or control sample were added to each capillary with a final reaction volume of 20 µl. The prepared capillaries were centrifuged with the adapters in the cold block at 2,000 g for 15 s. The PCR was performed according to the protocol given in Table 2. The Light Cyclor 2.0 software was used for the presence-absence data analysis and the quantitation was determined by channel 530 using the absolute quantitation second derivative analysis.

Table 1. Sequences of primary probes (Lam-Himlin et al. 2011).

GENE	Gene forward primer sequence (Tm °C)	Reverse primer sequence (Tm °C)	Amplicon size (Base pairs)
PDC	5'AGCGGTTGCAGCGACAA TAGGA3' (59)	5'CTGCAACCAGCGCTGCACCT3' (60)	149
Terminal 16S rRNA	5'CACACCGCCCGTCACAC CAT3' (59)	5'TGATCCAGCCGCGAGTTCTCTCT3' (59)	140

Table 2. Real-time PCR protocol for *S. ventriculi*.

Program	Denaturation	Amplification			Cooling
Analysis mode	No	Quantification mode			No
Loop	1	40			1
Target (°C)	95	95	55	72	40
Duration (hh:mm:ss)	00:10:00	00:00:10	00:00:30	00:00:01	00:00:30
Ramp rate (°C/s)	20	20	20	20	20
Acquisition Mode	No	No	No	No	No

Results

Real-time PCR analysis of 43 abomasum tissues obtained from lambs presenting bleeding, ulcer, gas or these lesions simultaneously in the abomasum at necropsy and of 6 healthy abomasum tissues was performed using primers targeting the terminal 16S rRNA gene of *S. ventriculi* and determined positivity in 11 cases, whereas the real-time PCR using primers targeting the *S. ventriculi* PDC amplicon determined positivity in 17 cases. In all of the healthy abomasum samples, negative results were obtained in the real-time PCR examinations for both target genes. The graph of the amplification curves obtained in real-time PCR examinations using primers targeting the PDC amplicon of cases in which *Sarcina*-like agents were detected by microscopic examinations is given in Fig. 1 (Plate I). No amplification using primers targeting the PDC amplicon could be detected in the examinations of the abomasum samples of the 6 healthy lambs, which were considered as controls in microscopic examinations (Plate I, Fig. 2).

The distribution of haemorrhagic abomasitis determined during the study and *S. ventriculi* diagnosed by real-time PCR in these cases are presented according to months in Table 3. At necropsy, it was macroscopically identified that the abomasa were greatly enlarged owing to gas accumulation, due to which a rupture occurred in 2 of them; abomasa shifted from the linear line in 24 cases as a result of the accumulated gas; the abomasum wall was thicker in 15 cases and thinner in 10 cases. In 5 cases, it was determined that the abomasum wall contained gas bubbles of different sizes, and haemorrhages ranging from petechiae to ecchymosis were found in the abomasum in all of the cases. Ulcer formations of different intensities were noted in 35 of the examined abomasa. Another remarkable finding in the study was the presence of hairballs of up to the size of a walnut, which was noticed in 22 cases (Plate II, Fig. 3B).

Table 3. Distribution of haemorrhagic abomasitis identified during the study and *Sarcina ventriculi* diagnosed in these cases by months.

	Haemorrhagic abomasitis	Real-time PCR		Microscopic <i>Sarcina</i> -like bacteria
		PDC amplicon	Terminal 16S rRNA	
January	6	2	1	
February	18	10	7	2
March	13	5	3	1
April	3			
May	1			
July	1			
August	1			
Total	43	17	11	3

*No animals with abomasitis were brought in between September and December and in June.

In the cases in which *S. ventriculi* was identified by real-time PCR, it was determined that the abomasum was enlarged and displaced in all cases; its wall was thickened in 10 cases, and thinned in 6 cases. It was observed that the wall contained gas bubbles of different sizes in 4 cases (Plate II, Fig. 3B). In addition, in all the cases with *S. ventriculi*, bleeding of varying severity from petechiae to ecchymosis was found in the abomasum (Fig. 3A). In two cases, it was determined that there was a rupture of a diameter of 1–2 cm in the abomasum wall, and due to this, the bloody contents of the abomasum spilt into the abdominal cavity. Numerous different sized ulcers were noted on the mucosa in all abomasa (Fig. 3B).

In all of the abomasa examined microscopically, different sized-areas of hyperaemia and bleeding, necrosis and shedding of the mucosal epithelium, and inflammatory cell infiltrations were determined. While oedema in the submucosa was detected in 32 cases, microscopic ulcers and neutrophil granulocytes located on the edges and over the ulcers were noticed in 25 cases. Gas bubbles located in the submucosa were seen in 5 cases. In 28 cases, mixed bacterial aggregations ranging from cocci to bacilli were encountered in the microscopic examination of the preparations stained with haematoxylin and eosin.

In the microscopic examination of the abomasum of the cases in which *S. ventriculi* was determined by real-time PCR, hyperaemia and bleeding areas of different sizes (Plate II, Fig. 4 A–B), necrosis and desquamation in the mucosal epithelium, ulcers and oedema in the submucosa were determined (Fig. 4 A–B). Neutrophil granulocytes located on the edges and on the ulcers were noticed. Gas bubbles settled in the submucosa were observed in four cases (Fig. 4A), while the microscopic examination of hematoxylin and eosin stained preparations revealed bacteria in a packed quadruple structure in 3 cases (Fig. 4D).

Discussion

Haemorrhagic abomasitis is mostly related to *Clostridium* spp. (Benavides et al. 2015; Sheehan 2020). However, some published studies show that, in addition to *Clostridium* spp., *Sarcina* spp. is effective in the aetiology of haemorrhagic abomasitis (DeBey et al. 1996; Vatn et al. 2000; Van Kruiningen et al. 2009). Agents such as *C. septicum*, *C. perfringens* type A, *C. sordellii* and *Sarcina* spp., alone or in combination, are known as the most common haemorrhagic abomasitis agents (Vatn et al. 2000; Prescott et al. 2016). In this study, *S. ventriculi* was determined by real-time PCR in 17 of the 43 haemorrhagic abomasitis cases examined. No studies have been conducted on clostridial agents.

It has been reported that abomasitis in lambs mainly occurs in 1–6-week-old animals and peaks at 3–4 weeks (DeBey et al. 1996). In this study, according to the calculations made considering the regional birth season, it was determined that all of the haemorrhagic abomasitis cases that composed our material were diagnosed in 2–6-week-old lambs. The results were in agreement with the literature.

Sarcina species has been reported to be a causative agent in abomasal swelling and death of livestock (DeBey et al. 1996; Van Kruiningen et al. 2009; Leite Filho et al. 2015). *Sarcina* species can survive even at low pH. They cause bloating due to excessive carbon dioxide production as a result of fermentation products and abomasal rupture due to excessive bloating (Edwards et al. 2008). Similar to the literature, abomasal swelling was noticed in all the cases with *S. ventriculi* detected by real-time PCR and rupture in the abomasum in 2 cases.

Vatn et al. (2000) reported that *Sarcina*-like bacteria were seen in 94% (44/47) of lambs with abomasal gas and 45% (9/20) of lambs with ulcers and/or bleeding. In this study, the rate of *Sarcina* determined by real-time PCR was 39.5%. Again, the fact that tetrad structures were seen in only 3 cases in microscopic examinations is lower than the rate reported by Vatn et al. (2000). Findings such as gas accumulation, bleeding, and ulcer in the abomasum determined by Vatn et al. (2000) were observed in all of the cases with *S. ventriculi* in our study, which is in line with the findings of Vatn et al. (2000). Considering the findings, a strong positive correlation between *Sarcina*-like bacteria and gas accumulation was appointed. The same researchers have reported that bezoars are often found in lambs with *Sarcina* species. Similarly, bezoars of different sizes were found in the abomasum of six lambs in our study (Fig. 3B).

Sarcina spp. are spherical-shaped bacteria with a diameter of 1.8 to 3.0 μm , seen in packages of 4 or more (Canale-Parola and Wolfe 1960; Canale-Parola 1986). The characteristic quadruple packing of *Sarcina* species is the result of cell division formed in at least two growth planes (Canale-Parola and Wolfe 1960; Canale-Parola 1970). *Micrococcus* spp. make Gram-positive cocci clumps that appear in bundles like *Sarcina* spp. (Hubalek 1969). In microscopic examinations, they can be mistaken for *Sarcina* species at first glance. However, in distinguishing the two organisms, it is important that *Micrococcus* species are considerably smaller than *Sarcina* species, with an average size of 0.5 μm , and *Micrococcus* species form different clusters compared to *Sarcina* species. Although *Sarcina* species have different microscopic features, such as the size and shape of aggregation, the causative agent must be specifically identified for a definitive diagnosis.

The PDC gene is responsible for the conversion of pyruvate to acetaldehyde and carbon dioxide in *S. ventriculi*. This amplicon was not detected in any of the 6 healthy abomasum tissues examined in the study (Fig. 2). While the result of real-time PCR examination performed with primers targeting terminal 16S rRNA was positive in 11 cases, positivity was determined in 17 cases with primers targeting the PDC amplicon, which we selected as evidence for the presence of *S. ventriculi* (Fig. 1). Although our results indicate that the PDC gene can be used in the diagnosis similarly to the previous study Lam-Himlin et al. (2011), we think that the diagnosis should be supported by macroscopic and microscopic findings.

The cases of haemorrhagic abomasitis caused by *Sarcina* species are probably not a new condition in lambs. However, the routine use of molecular methods has begun to show the true extent of the agent. In this study, the haemorrhagic abomasitis cases related to *S. ventriculi* determined by real-time PCR in 17 lambs are important as it is the first study conducted in our country. In order to elucidate the role of *Sarcina* species in abomasitis, detailed studies are needed to investigate other haemorrhagic abomasitis agents and to evaluate nutritional programs.

In this study, histopathological findings were determined in haemorrhagic abomasitis cases in which *Clostridium* spp. could not be determined by specific immunohistochemical staining and in abomasum samples whose gene regions specific to *Sarcina* spp. were determined indicate that *Sarcina* spp. can be considered as an aetiological agent. The real-time PCR analyses and histopathological examinations of samples obtained from the abomasum will help diagnose abomasitis caused by *Sarcina* species.

In this study, the role of *S. ventriculi* in the aetiology of haemorrhage, ulcer, and gas in the abomasum or their coexisting abomasitis cases at necropsy in lambs was revealed by histopathological and real-time PCR methods, while the optimization of the real-time PCR method for the diagnosis of haemorrhagic abomasitis caused by *S. ventriculi* was accomplished.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Aubry P 2004: Abomasitis associated with *Sarcina*-like organisms in young dairy calves, in Proc 23rd World Buiatric Congress, Quebec, Canada Canadian Association of Bovine Veterinarians, 34 p.
- Benavides J, González L, Dagleish M, Pérez V 2015: Diagnostic pathology in microbial diseases of sheep or goats. *Vet Microbiol* **181**: 15-26
- Canale-Parola E, Wolfe R 1960: Studies on *Sarcina ventriculi* II. Nutrition. *J Bacteriol* **79**: 860
- Canale-Parola E 1970: Biology of the sugar-fermenting Sarcinae. *Bacteriol Rev* **34**: 82
- Canale-Parola E 1986: Genus *Sarcina*. In: Bergey's Manual of Systematic Bacteriology, Vol. 2, P H A Sneath, N S Mair, M E Sharpe, J G Holt, Eds, The Williams and Wilkins, Baltimore, pp. 1100-1103
- Çiftçi M, Ortatath M, Erer H, Hatipoğlu F, Özdemir Ö 2015: Veteriner Sistemik Patoloji, 2nd edn, SÜ Basımevi, Konya, 230 p.
- DeBey BM, Blanchard PC, Durfee PT 1996: Abomasal bloat associated with *Sarcina*-like bacteria in goat kids. *J Am Vet Med* **209**: 1468-1469
- Dillard K, Anttila M 2005: *Sarcina*-like bacteria in a calf with abomasitis and abomasal tympany: a case report. *Suom Eläinlääk* **111**: 72-75
- Edwards G, Woodger N, Barlow A, Bell S, Harwood D, Otter A, Wight A 2008: *Sarcina*-like bacteria associated with bloat in young lambs and calves. *Vet Rec* **163**: 391-393
- Hubalek Z 1969: Numerical taxonomy of genera *Micrococcus* Cohn and *Sarcina* Goodsir. *Microbiology* **57**: 349-363
- Lam-Himlin D, Tsiatis AC, Montgomery E, Pai RK, Brown JA, Razavi M, Lamps L, Eshleman JR, Bhagavan B, Anders RA 2011: *Sarcina* organisms in the gastrointestinal tract: a clinicopathologic and molecular study. *Am J Surg Pathol* **35**: 1700
- Leite Filho RV, Bianchi MV, Fredo G, Oliveira ECd, Laisse CJM, Driemeier D, Pavarini SP 2015: Emphysematous abomasitis in a lamb by bacteria of the *Sarcina* genus in Southern Brazil. *Ciência Rural* **46**: 300-303
- Lewis C 2007: Clostridial diseases. In: Aitken ID (Ed): Diseases of sheep. Blackwell Publishing, Oxford, UK. Fourth edn. 647 p.
- Luna L 1968: Routine staining procedures. Manual of histologic staining methods of the Armed Forces Institute of Pathology. Blakiston Division McGraw-Hill, New York, 258 p.
- Prescott JF, Menzies PI, Fraser RS 2016: 17 Clostridial Abomasitis. In: Uzal FA, Songer JG, Prescott JF, Popoff MR (Eds): Clostridial Diseases of Animals. John Wiley & Sons, Inc. 336 p.
- Schemm T, DeBey B, Nagaraja TG 1999: *Sarcina ventriculi* as the potential cause of abomasal bloat. *Kansas Agric Exp Stn Res Reports*, **1**: 130-131
- Sheehan M 2020: Clostridial Diseases in Bovine and Ovine. All-Island Animal Disease Surveillance Report 2020. Department of Agriculture, Food and the Marine of Ireland, Agri-Food and Bioscience Institute and, Animal Health Ireland. 176 p.

- Van Kruiningen HJ, Nyaoke CA, Sidor IF, Fabis JJ, Hinckley LS, Lindell KA 2009: Clostridial abomasal disease in Connecticut dairy calves. *Can Vet J* **50**: 857-860
- Vatn S, Tranulis M, Hofshagen M 2000: Sarcina-like bacteria, *Clostridium fallax* and *Clostridium sordellii* in lambs with abomasal bloat, haemorrhage and ulcers. *J Comp Pathol* **122**: 193-200

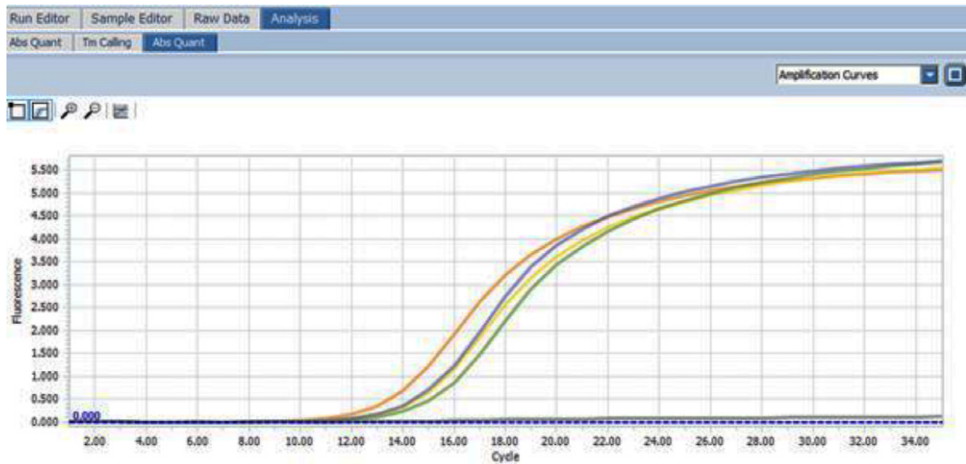


Fig. 1. Amplification curves obtained in real-time PCR examinations using primers targeting the PDC amplicon of cases with *Sarcina*-like agents in microscopic examinations.

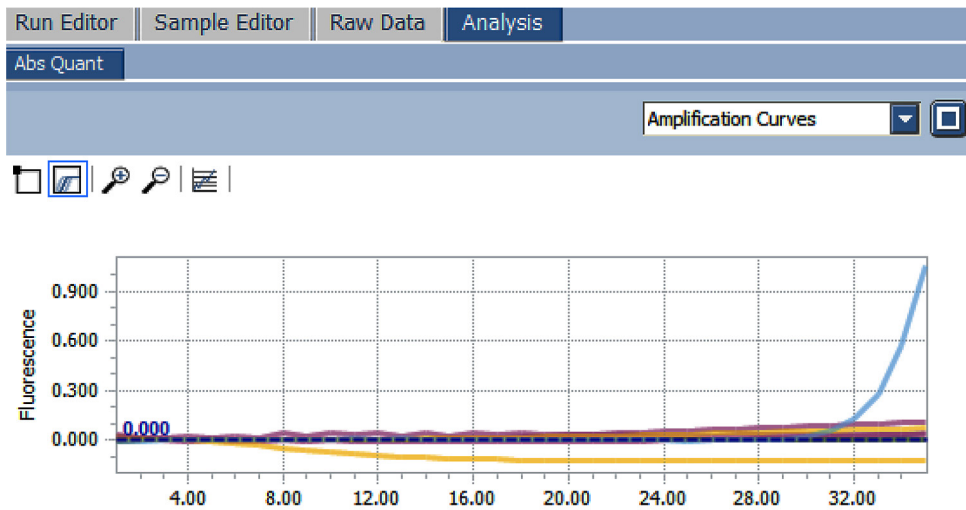


Fig. 2. Amplification curves obtained in real-time PCR examinations using primers targeting the PDC amplicon of abomasum samples of healthy lambs evaluated as a control in microscopic examinations.

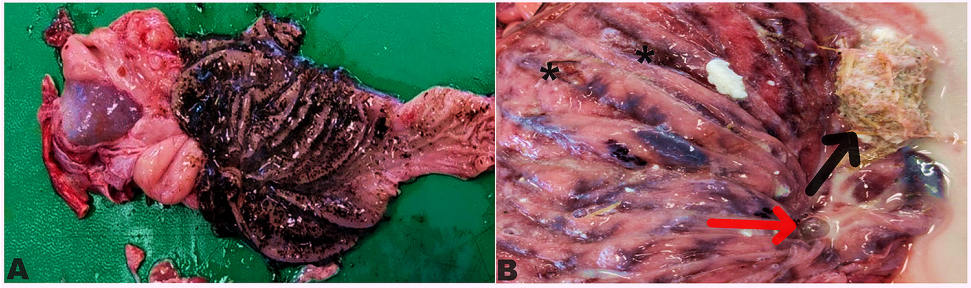


Fig. 3. Macroscopic examination. A: Intense bleeding foci in the abomasum mucosa. B: Ulcer (star), bezoar (arrow) and gas bubble (red arrow) in the abomasum mucosa.

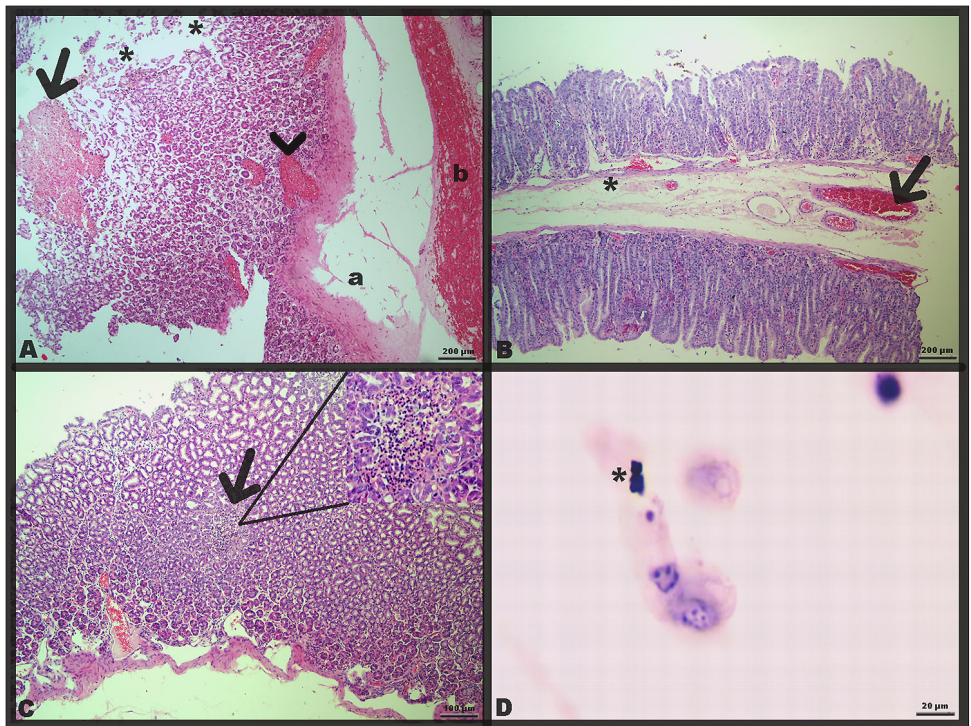


Fig. 4. Histopathological examination, haematoxylin and eosin. A: Degeneration of the abomasum mucosal epithelium, desquamation (stars), bleeding focus (arrow), hyperaemia in the proprial layer (arrowhead), gas (a) and bleeding (b) in the submucosa. B: Oedema (star) and hyperaemia (arrow) in the submucosa. C: Inflammatory cell infiltrates in the abomasum mucosa (arrow). D: Sarcina-like agent (star) in packaged style.