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Brief Communication

Possible Involvement of *Sarcina ventriculi* in Canine and Equine Acute Gastric Dilatation

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Acute gastric dilatation (AGD) has been reported from man, dogs, horses, monkeys, and ruminants, among other species (*Van Kruiningen et al.* 1974). In the dog, several predisposing and etiological factors have been discussed, including breed, weight, feeding patterns, bacterial fermentation, and aerophagia (*Van Kruiningen et al.* 1974, *Caywood et al.* 1977, *Rogolsky et al.* 1978, *Glickman et al.* 1994). Nasal mite infections, causing reversed sneezing and supposedly aerophagia, has also been associated with AGD (*Bredal* 1998). In horses, grain engorgement is assumed to be important in the pathogenesis (*Van Kruiningen et al.* 1974).

Acute abomasal dilatation, or bloat, has been reported in preruminant lambs, and in 44 out of 47 affected lambs numerous *Sarcina*-like bacteria were seen on unstained smears, identified to be *Sarcina ventriculi* in one lamb (*Vatn et al.* 2000). Based on these findings, a preliminary study was carried out to examine gastric contents of other species for the presence of *S. ventriculi* or *Sarcina*-like bacteria. In this paper we report the findings in 2 dogs and one horse with AGD, compared to 10 canine and 5 equine controls.

Routine necropsy procedures were applied on all animals. Gastric contents and mucus were examined by phase contrast microscopy of unstained smears (wet mounts x 320). Based on bacteriological findings in lambs, emphasis was put on anaerobic cultivation for *S. ventriculi* and *Clostridium* spp. (*Vatn et al.* 2000). Gastric contents from the cases were inoculated on 5% sheep blood agar plates and in deoxygenated Sarcina growth medium (*Atlas* 1993) with a pH of 2.2, and incubated anaerobically at 37°C overnight. After 2 more enrichments, the culture was plated on Sarcina medium with a pH of 6.0, to which 15% Bacto-Agar (Difco, Detroit, USA) had been added. Sugar fermentation for the identification of *S. ventriculi* was carried out according to *Crowther* (1971). Extracellular cellulose was detected by adding colonies from overnight cultures to a 100 mM citrate buffer, pH 4.0, containing 1% cellulase (Merck, Darmstadt, Germany), incubated at 37 °C overnight and evaluated by disruption of the *Sarcina* bundles. Colonies suspected of being *Clostridium* spp. (no growth under aerobic conditions, catalase negative, gram-positive rods) were tested on API rapid 32 A (bioMérieux, Marcy-l'Etoile, France) according to the producer's manual.

Tissue samples for histopathology were fixed in 10% neutral buffered formalin, processed routinely and stained with HE.

Case No. 1 (dog): A three-year-old female English mastiff was found dead in the morning, without previous symptoms, and necropsied later the same day. The dog was on oral antibiotic treatment (cefalexin) due to mastitis. Large amounts of gas were present in the dilated stomach, which showed a 360° volvulus including the anterior parts of the small intestines. The spleen was enlarged.

Case No. 2 (dog): This two-year-old male of mixed breed, with a weight of 42.5 kg, was also found dead in the morning, without previous symptoms, and was necropsied one day later. The dog had been treated for gastric dilatation 3 weeks earlier. Post-mortem findings resembled those of case No. 1, but with a 180° volvulus.

Case No. 3 (horse): A 5-year-old, female cold-blooded trotter was presented with signs of severe acute colic and reflux. The horse did not respond to pain treatment (metamizol) and was euthanased. At necropsy, 12 h later, a post-mortem rupture of the stomach was present, and the jejunum and ileum were expanded and filled with gas and watery contents. Large amounts of parasites (*Cyathostomes* spp.) were found in the colon, and small erosions and ulcers were found around the ileocaecal valve.

Unstained smears from both cases Nos. 1 and 2 showed numerous *Sarcina*-like bacteria (≥ 5 bundles per field, magnification x 320) in the mucus and gastric contents (Fig. 1). Gram staining of the smears complicated the evaluation, as the gram-positive *Sarcina*-like bacteria appeared as dark, dense structures. Cultivation from case No. 1 yielded *S. ventriculi*, which did not grow under aerobic condition, and was catalase negative, possessed extracellular cellulose and stained gram-positive. It was able to ferment glucose, fructose, galactose, lactose, maltose, and sucrose, but not L-arabinose, dulcitol, glycerol, mannitol, salicin or xylose. Raffinose was fermented after 2 days and weak growth was seen in broth containing inulin. The results correspond to earlier findings (*Crowther* 1971, *Canale-Parola* 1986, *Vatn et al.* 2000). On agar plates the bacteria formed large white colonies with a bulgy surface, and in one colony holes were formed, presumably due to the escaping gas (Fig. 2). Selective cultivation for *S. ventriculi* from case No. 2 was unsuccessful. Direct microscopy of gastric contents and mu-

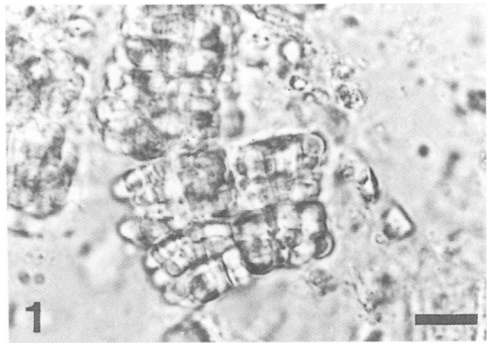

Figure 1. Gastric contents from case No.1, dog with AGD. Unstained smear with several *Sarcina*-like bacteria, where each packet consists of a large number (>50) of cocci. Bar = 10 μm.

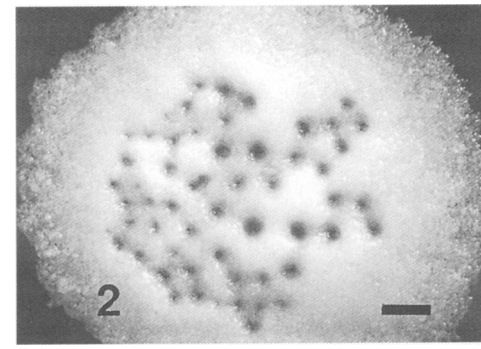

Figure 2. Bacterial colony of *Sarcina* ventriculi isolated from gastric contents of a dog with AGD. The holes in the colony are presumably caused by vigorous gas production. Bar = 500 μm.

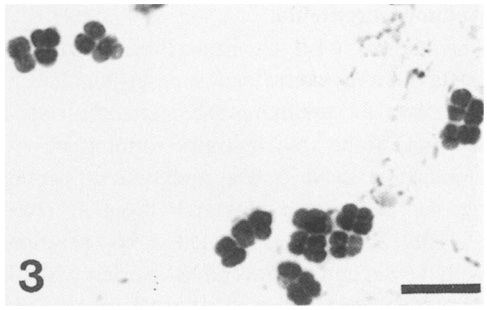

Figure 3. Gastric mucus from case No. 3, a horse with AGD. Section showing Sarcina-like bacteria in the mucus. HE. Bar = 10 μm.

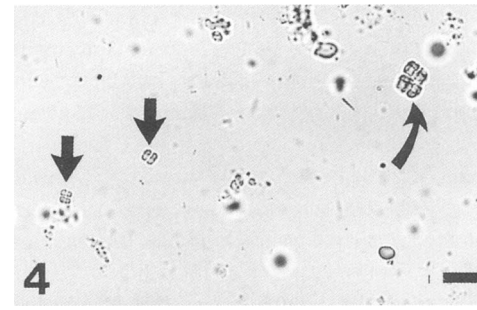

Figure 4. Gastric contents from the one positive control dog. *Sarcina*-like bacteria consisting of 4 (straight arrow) to 16 (curved arrow) visible cells. Bar = 25 μm.

cus from case No. 3, revealed more moderate amounts of *Sarcina*-like bacteria, some of which resembled those seen in cases Nos. 1 and 2, but also smaller packets of only 2, 4 or 8 cocci. Cultivation for *S. ventriculi* was negative. For all 3 cases, evaluations of sections were not rewarding due to loss of mucus, but in case No. 3 scattered *Sarcina*-like bacteria were seen on the mucosal (Fig. 3) and serosal side. Anaerobic cultivation from the gastric contents on sheep blood agar plates yielded growth of *Clostridium sordellii* (99.9% probability with t-value = 0.88 on the API rapid 32 A).

Sarcina-like bacteria were only found on smears and sections in one out of 10 canine controls, which were all necropsied between 1 and 24 h post mortem. The one positive dog had moderate amounts (0-2 bundles per field) of *Sarcina*-like bacteria in the contents, and most of these consisted of smaller conglomerates of 2 to 8 cocci (Fig. 4), and only very few resembled the large packets seen in cases Nos. 1 and 2. The 5 equine controls all had scattered *Sarcina*-like bacteria in the gastric contents, resembling those seen in case No. 3, but in slightly lower numbers.

This is the first report demonstrating the presence of numerous *S. ventriculi* in association with AGD in monogastric animals. The morphology and the amounts of *Sarcina*-like bacteria in cases Nos. 1 and 2 were equivalent to that demonstrated in lambs with abomasal bloat (*Vatn et al.* 2000), and also in a herd outbreak in goat kids (*DeBey et al.* 1996). Case No. 3 had slightly more *Sarcina*-like bacteria as compared to the equine controls, but the role of the bacteria in this horse is debatable. It is interesting though, to note that large numbers of «Sarzinen», containing 4 cocci in each bundle, were demonstrated in the aspirate from the peritoneal cavity of a horse with a ruptured stomach (*Mocsy* 1954).

It is, however, unknown whether the different sizes of the *Sarcina* bundles indicate different levels of bacterial metabolism, or different bacterial species. Possibly the smaller bundles represent a more inactive form of the bacteria, as opposed to the large bundles (>50 cocci) seen in pure culture and in contents from animals with acute gastric dilatation. However, *Sarcina maxima*, which is the only other species belonging to the genus *Sarcina*, has a similar morphology but forms smaller bundles, and has been isolated from horse faeces (*Canale-Parola* 1986).

S. ventriculi is ubiquitous and has been isolated

from soil and mud. The bacteria was first described by *Goodsir* in 1842, in a young man with gastric fermentation, but has later been found in faeces of healthy humans (*Crowther* 1971), and bacteria supposed to be *S. ventriculi* were described in 2 healthy monkeys (*Ohwaki et al.* 1974). As *Sarcina*-like bacteria seem to be part of the normal gastric flora of horses but not of dogs, lambs, or goat kids (*DeBey et al.* 1996, *Vatn et al.* 2000), differences in host species seem to exist.

S. ventriculi is extremely pH tolerant and may outgrow other bacteria in the stomach, if sufficient fermentable carbohydrates are available (*Canale-Parola* 1986). Its vigorous production of CO_2 may explain the elevated levels of this gas demonstrated in some dogs with AGD (*Van Kruiningen et al.* 1974, *Rogolsky et al.* 1978). In both lambs and dogs a foamy surface and a continuing fermentation of the gastric liquid have been observed, indicating that the gas was produced in the stomach (*Van Kruiningen et al.* 1974, *Vatn et al.* 2000).

C. sordellii has been isolated from lambs with abomasal disorders (*Lewis et al.* 1998, *Vatn et al.* 2000), but further studies are needed to evaluate the significance of the isolation of *C. sordellii* from case No. 3, as no cultivation was undertaken in the equine controls.

We believe that *S. ventriculi* was a major contributor to the gas production in the 2 dogs with AGD (cases Nos. 1 and 2), and that *S. ventriculi* or *Sarcina*-like bacteria should be considered in future studies of the etiology of AGD in various species. Further work is needed to establish better cultivation methods for *Sarcina* spp. from stomach contents, but so far the evaluation of unstained smears seem to be the most reliable and simple diagnostic method.

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