**FULL PAPER** *Surgery*

# A Mechanism of Excessive Accumulation of Abomasal Gas in Vagotomized Cattle Determined using Fluoroscopy

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ABSTRACT. To better understand the mechanism of excessive gas accumulation in the abomasum in bovine abomasal displacement, we performed gastric fluoroscopy in vagotomized cattle. Fifteen 6-month-old Holstein steers were divided into three groups: a non-vagotomized control group (Group C; n=5), a ventral thoraco-vagotomized group (Group V; n=5), and a dorsal and ventral thoraco-vagotomized group (Group DV; n=5). These groups were examined by fluoroscopy before and during a 5-week observation period after surgery. In Group C, no change was observed throughout the observation period. In Group DV, immediately after surgery, reticuloruminal motility was completely absent and ruminal distention was seen. Two weeks after surgery, abnormal reticulum motility and increased gas accumulation in the abomasal body were noted. Abomasal dilatation was also observed. In Group V, 1 week after surgery, gas inflow into the abomasum and relatively normal reticulum motility were observed along with a rapid increase in abomasal gas. Abomasal dilatation was also observed. In addition, left-displaced abomasum occurred in one of the steers in this group. From these results, we concluded that one of the mechanisms of excessive gas accumulation in the abomasum is reticulum-mediated gas inflow from the rumen combined with vagotomy-induced hypomotility.

KEY WORDS: abomasum gas, cattle, displaced abomasum, vagotomy.

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Displaced abomasum (DA) is one of the periparturient disorders increasingly seen in dairy cattle during the past quarter century. Its etiology is multifactorial [7], reportedly involving increased levels of volatile fatty acids in the rumen and abomasum [26], hypocalcemia [4, 16, 20, 22], negative energy balance [13 23], metabolic alkalosis [19], prostaglandins [3], vagus nerve injury [17, 24, 27, 28], and nitroxidergic and vasoactive intestinal peptide-containing nerves in the muscular layer of the abomasum [9, 21]. However, none of these factors have been established by experimental work, and the exact mechanism of displacement remains largely unknown.

Two pathogenic factors are crucial for the development of DA: abomasal atony with hypomotility is the essential prerequisite [5, 8, 24], while excessive accumulation of abomasal gas is the absolute requirement [25]. As for the former requirement, Soehartono *et al.* [24] have proposed that decreased intramural innervation of the abomasum due to vagal nerve injury is one of the etiological factors in abomasal atony with hypomotility, based on the radiographic and immunohistochemical findings in calves that underwent total thoraco-vagotomy. On the other hand, no study has reported the mechanism of abomasal gas accumulation to date. To better understand how excessive gas accumulation occurs in DA, we performed thoraco-vagotomy in 6-month-old cattle with a morphologically and functionally developed complex stomach system [1] and examined radiographic changes in the reticulum and abomasum over time in comparison with those in control cattle of the same age.

## MATERIALS AND METHODS

Fifteen 6-month-old Holstein steers (body weight: 195 ± 21 kg) produced and raised at the Hokkaido Animal Research Center were used for the study. We divided these animals into three study groups: a ventral thoraco-vagotomized group (Group V; n=5), a dorsal and ventral thoraco-vagotomized group (Group DV; n=5), and a non-vagotomized control group (Group C; n=5). Steers were housed in tie stalls for 10 days prior to vagotomy and fed 6 kg dry matter (DM) of corn silage before the vagotomy, and 2 kg DM of corn silage after the vagotomy, once a day at 9:30 am. Water and salt block were provided ad libitum. Feed was withheld for 48 hr before surgery.

Anesthesia was induced by intravenous injection of 0.1 mg/kg xylazine (Emasus® 2% injection, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan). The trachea was then intubated and the animal was held on a surgical table in right lateral recumbency. Anesthesia was maintained by inhalation of isoflurane (ISOFLU®, Dainippon Sumitomo Pharma Co., Ltd., Osaka) and oxygen. The surgical area was aseptically prepared and thoracotomy was performed by resecting the left 7th and 8th ribs. In Group V, the ventral vagus trunk immediately cranial to the diaphragm was separated from the esophagus, and a 3-cm section was removed. In Group DV, both the ventral and dorsal vagus trunks were cut similarly. The chest was closed in the usual manner. Animals in Group C were sham-operated; thoracotomy and rib resec-

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tions were similarly performed, and the chest was closed. For 1 week after surgery, 10 m*l* of an aqueous dihydrostreptomycin sulfate/procaine benzylpenicillin combination (Mycillin® Sol, Tamura Seiyaku, Tokyo, Japan) was administered intramuscularly every morning. When dehydration or any other abnormal condition was suspected from hematocrit or total blood protein values, 2 l of 1/2 Ringer's + 5% glucose solution (Isotonic Ringer's Solution with Glucose, Nippon Zenyaku Kogyo, Fukushima, Japan) was infused intravenously. When severe distention of the rumen was observed, gas was removed through a long needle (18 gauge × 70 mm hypodermic needle, NOPRO, Tokyo) inserted in the flank.

Physical examination and fluoroscopic observation of reticulum contraction, abomasal gas accumulation, and abomasal dilatation were performed at 9:00 am before surgery and at 2 and 4 days and 1, 2, 3, 4, and 5 weeks after surgery. During physical examination, ruminal gas accumulation was scored on a 4-point scale (bloat score) based on the appearance and palpation of the left flank: 0 for lack of distention of the left flank and normal respiration; 1 for distended but resilient flank and normal respiration; 2 for distended, tense flank and mild tachypnea; and 3 for distended, tense flank, and tachypnea requiring ruminal puncture. Fluoroscopic observation was performed using an X-ray system (320 kV; MG325, Philips Electronics, Tokyo) and an X-ray image intensifier (IT-12HTL, Hitachi Medical, Tokyo). Captured images were stored in movie and image file formats for further analysis. Reticular contraction was assessed by the duration (sec) of the interval between biphasic contractions and the difference in the level (cm) of the reticular fundus between the non-contracting and maximum contraction phases (contraction amplitude). Abomasal gas content was estimated from the radiolucent area ($cm^2$), when gas accumulation at the lesser curvature of the abomasal body reached a maximum. The degree of abomasal dilatation was estimated from the length (cm) of the abomasum along the fundus, which is an indicator of abomasal atony [24]. The area and length were calculated using Lenaraf ver. 2.20 software (Atelier M&M, Chiba, Japan) for Microsoft Excel. Values for bloat score are presented as median (minimum maximum), others are presented as means ± SD and analyzed by the Kruskal-Wallis test and Scheffe's or Steel's multiple comparison test. P values less than 0.05 were considered statistically significant.

All experiments were conducted in accordance with the animal ethics guidelines at Obihiro University of Agriculture and Veterinary Medicine.

Because the dorsal vagus nerve has little effect on abomasal motility [6, 12, 14], we did not perform dorsal-only thoraco-vagotomy.

## RESULTS

In Groups C and V, no changes occurred in the appearance of the left flank throughout the study period, and bloat score was 0 in all animals at all time points. In Group DV, on the other hand, the scores increased after surgery. Peak scores were observed 1–3 weeks after surgery (median score, 1–2). Three steers had a score of 3; one at 4 days, one at 1 week, and one at 2 weeks after surgery, and a "papple" shape was evident from behind in these steers. In Group DV, distention of the left flank was apparent in all animals from 1 to 2 weeks after surgery, and bloat score was significantly higher than that in Groups C and V ($p<0.01$).

The amplitude of reticular contraction is shown in Fig. 1. In Group C, this remained unchanged throughout the study period. In Group V, the contraction amplitude decreased by almost 50% at 2 days after surgery but recovered to the preoperative level by 2 weeks after surgery. In Group DV, reticular motility ceased completely after surgery. Weak reticular contraction resumed at 2 weeks after surgery, but it was merely an intermittent, vertical movement rather than the characteristic biphasic contraction. There was no change in contraction interval of the reticulum before or after surgery in Group C (59.0 ± 22.4 sec) or Group V (54.7 ± 7.6 sec). In Group DV, the interval could not be measured.

Maximum areas of accumulated gas in the abomasum are plotted in Fig. 2. In Group C, the amount of abomasal gas changed only minimally and remained between 13.6 and 26.6 $cm^2$ throughout the study period. In group V, the amount of abomasal gas increased sharply 1 week after surgery (152.7 ± 230.2 $cm^2$); it decreased thereafter but remained higher than in the other groups. At 1 week after surgery, in one of the 5 steers in Group V, the "ping test" was positive on the left abdomen, and abomasum dilatation, gas accumulation and displacement was observed on fluoroscopic images. The steer developed left displaced abomasum (LDA), which was corrected by rolling at 8 days after surgery, no recurrence was observed. In Group DV, abomasal gas accumulation was not evident until 1 week after surgery, but the amount of gas increased to a maximum of

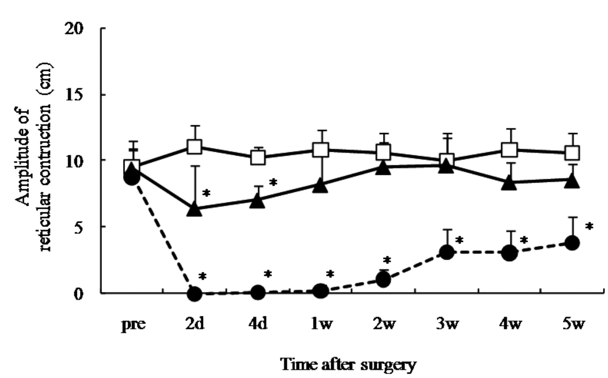


Fig. 1. Mean reticular contraction amplitude ± SD (cm) before and during the 5-week observation period in the non-vagotomized control group (□, Group C; n=5), ventral thoraco-vagotomized group (▲, Group V; n=5), and dorsal and ventral thoraco-vagotomized group (●, Group DV; n=5). Mann-Whitney U test was used to determine significant differences between values. * Significant difference between Group C and Group D or between Group C and Group DV ($p<0.05$).

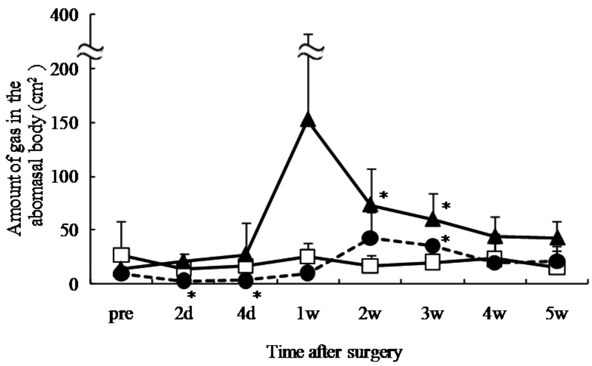


Fig. 2. Mean gas areas in the abomasal body (cm$^2$) ± SD before and during the 5-week observation period in the non-vagotomized control group (□, Group C; n=5), ventral thoraco-vagotomized group (▲, Group V; n=5), and dorsal and ventral thoraco-vagotomized group (●, Group DV; n=5). Mann-Whitney U test was used to determine significant differences between values. * Significant difference between Group C and Group D or between Group C and Group DV ($p<0.05$).

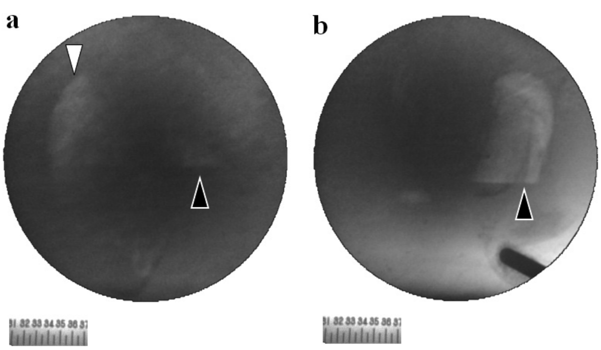


Fig. 3. Fluoroscopic images of the abomasal body. (a) First, gas was observed in the omasum (white arrowhead), and there was little gas in the abomasum (black arrowhead). (b) After reticular contraction, the gas in the omasum disappeared, and gas appeared in the abomasum (black arrowhead). Scale unit, 0.5 cm.

42.9 ± 24.3 cm$^2$ at 2 weeks after surgery.

Abomasal gas fluctuated in amount; increases in gas volume were observed simultaneously with reticular contractions. At each reticular contraction, gas observed along the omasum lining disappeared, and the amount of abomasal gas increased (Fig. 3).

Figure 4 shows length of the abomasal fundus. In Group C, the abomasal length decreased with time after surgery; it tended to increase 4 days after surgery in Group V and 4 weeks after surgery in Group DV. Furthermore in group V, the abomasum was significantly longer than in Group C at 1 week after surgery. For the steer in Group V that developed DA, the data at 1 week were obtained after DA correction (9 days after surgery).

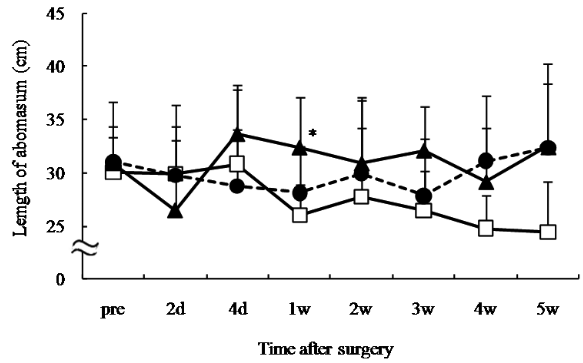


Fig. 4. Mean lengths of the abomasum ± SD (cm) before and during the 5-week observation period in the non-vagotomized control group (□, Group C; n=5), ventral thoraco-vagotomized group (▲, Group V; n=5), and dorsal and ventral thoraco-vagotomized group (●, Group DV; n=5). Mann-Whitney U test was used to determine significant differences between values. * Significant difference between Group C and Group V ($p<0.05$).

## DISCUSSION

Gastric motility in adult cattle is regulated extrinsically by the sympathetic and parasympathetic (vagus) nervous system and intrinsically by the enteric nervous system [15, 18]. In the thoracic cavity, the right and left vagus nerves pass over the heart and divide into dorsal and ventral branches, which unite with their counterparts to from the dorsal and ventral vagus trunks associated with the esophagus. The dorsal and ventral vagus trunks continue through the diaphragm. Branches from the dorsal vagus trunk supply mainly the rumen and reticulum, while those from the ventral vagus trunk distribute mainly to the reticulum, omasum, and abomasum. The balance between the activity of these vagal branches and that of the intrinsic submucosal plexus determines the motility of each gastric chamber [11]. In one study [2], thoracic dorsal and ventral vagotomy was performed in 2-week-old calves. This inhibited contractions in the pyloric part and body of the abomasum; leading to reduced abomasal motility; hence, the movement of ingesta from the abomasum to the duodenum was greatly retarded. Another study [24] reported that when 1-week-old calves underwent thoraco-vagotomy of both dorsal and ventral trunks, they immediately developed tonus, followed by atony after 1 week, and about half of them developed abomasal impaction. These studies have successfully induced abomasal atony with hypomotility, a prerequisite for the occurrence of DA, and vagus nerve injury was strongly suggested to be one of the etiological factors of DA, but not the sole cause of DA. We considered that thoraco-vagotomy alone did not cause DA in these studies because the stomach was not fully developed in these unweaned calves and thus the second factor required for DA, *i.e.*, accumulation of abomasal gas, was not present. In the present study, therefore, we aimed to investigate the mechanism of abomasal gas accumulation by performing vagotomy in 6-month-old cattle, which have morphologically and functionally developed stomachs [1].

In Group C, thoracotomy did not have a significant effect on the reticular and abomasal parameters. After surgery, the abomasal fundus shortened, but this was likely due to the reduced ingesta because the feed intake was reduced to one-third of the preoperative amount.

In contrast, the steers in Group DV developed distension and increased tension in the left flank as well as tachypnea; these findings were most severe at 1–3 weeks after surgery and were indicative of ruminal tympany. A significant difference in these clinical findings was observed 1–2 weeks after surgery between Group C and Group DV. We considered that both dorsal and ventral vagotomies led to gas retention or tympany in the rumen: ruminal hypomotility suppressed the eructation reflex, while loss of reticular motility (biphasic and triphasic contractions) caused failure in rumination and gas movement from the rumen to the abomasum. Reticular motility in these steers, however, returned gradually starting from 2–3 weeks after surgery, and this return coincided with a decrease in ruminal distension and abomasal gas accumulation. These results indicate that reticular contraction plays a crucial role in gas inflow from the rumen to the abomasum. It appears that reticular motility returned because it is controlled by the intrinsic submucosal plexus after vagotomy [10]. Abomasal dilatation did not occur in Group DV, and elongation of the abomasum occurred only after 4 weeks. It is likely that abomasum dilatation was not detected by fluoroscopy because the distended rumen was occupying the abdominal space, physically compressing the abomasum.

In Group V, the forestomach seemed to be functioning, because the interval between biphasic contractions of the reticulum was similar to that in Group C, although there was a slight decrease in contraction amplitude until 1 week after surgery. As gases accumulated rapidly in the abomasal body after that week, however, DA developed in one of the five steers. As mentioned earlier, dorsal and ventral thoraco-vagotomy in calves causes abomasal atony with hypomotility at 1 week after surgery [24]. Similarly, in the present study, the abomasal fundus started to lengthen 4 days after surgery in Group V, and became significantly longer than that in Group C at 1 week after surgery, indicating that “abomasal atony with hypomotility” was induced. In addition, we confirmed by fluoroscopy that reticular contractions caused disappearance of omasal gas and simultaneous appearance of abomasal gas. This observation suggests that abomasal gas accumulation was caused by gas inflow from the forestomach but not by gas production in the abomasum itself. In group V, the contraction of the reticulum, although weakened, sent the gases produced in the rumen into the abomasum. The abomasum, however, was atonic and hypomotile as a result of ventral thoraco-vagotomy. This seems to be the basis of excessive retention of abomasal gas, leading to the development of LDA 1 week after surgery in one of the five steers in Group V.

In conclusion, the present study experimentally induced excessive accumulation of gas in the abomasum in 6-month-old vagotomized cattle. Our findings indicate that this occurs as a result of gas inflows from the rumen into the abomasum via the reticular groove and the omasoabomasal opening. Reticular contractions seem to be essential for this gas movement. At the same time, abomasal atony with hypomotility suppresses gas movement into the duodenum, causing abomasal gas accumulation that ultimately leads to DA.

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