

## Assessment of Serum Pepsinogen and Other Biochemical Parameters in Dairy Cows with Displaced Abomasum or Abomasal Volvulus before and after Operation

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### Abstract

The objective of this study was to carry out epidemiological investigations in cows with displaced abomasum (DA) and to evaluate the recovery of abomasal condition by determining serum pepsinogen and other biochemical values. One hundred twenty-three cows with DA were used in this study. The occurrence rates within 3 weeks after parturition were 80.9% (72/89) for left displacement of the abomasum (LDA) and 65.0% (13/20) for right displacement of the abomasum (RDA), whereas 42.9% (6/14) of abomasal volvulus (AV) occurred after more than 4 weeks. The percentages of dead and disused animal were higher for cows with AV (28.6%) and LDA (23.6%) than for those with RDA (5.0%). There were remarkable increases in nonesterified fatty acids (NEFA) in all cows with DA at operation, whereas hyperglycemia was observed in cows with RDA and AV. The majority of cows with DA showed hypocalcemia and hypochloremia. There were no significant variations in the serum and blood biochemical parameters between free and tie stall cows with DA. The pepsinogen

value was markedly decreased in all cows with DA. Pepsinogen values in cows with LDA, RDA and AV at 7-11 days after operation were still lower than the reference value, although most biochemical parameters returned to the normal ranges post-operation. This finding indicates that the mucosa of the abomasum did not recover even at that time.

### Introduction

Displaced abomasum (DA) is a multifactorial disease. The disease is known to be associated with negative energy balance (NEB) and increased accumulation of triglycerides (TG) in the liver and is accompanied by other periparturient diseases (Muylle et al. 1990 and Geishauser, 1995). The prevalence of cows with DA has been recognized to be increasing because cows are being fed intensively on large farms (Cameron et al. 1998, Kelton et al. 1998 and Radostits et al. 2007). Displaced abomasum results in economic losses due to treatment and reduced milk production. In addition, it has been reported that 10% of cows with DA are culled or died (Bartlett et al. 1995 and Van Winden and Kuiper, 2003).

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Pepsinogen is a proenzyme, generated by chief cells of the abomasal mucosa. Hydrochloride secreted from parietal cells changes pepsinogen to pepsin (Vianello et al. 1988 and Tanaka et al. 1991). It has been reported that the serum pepsinogen value can be employed as a useful marker for assessing the pathogenesis of abomasal diseases (Berghen et al. 1993, Zadnik and Mesaric, 1999 and Mesaric et al. 2002). The serum pepsinogen value in healthy cows is not significantly changed with the lactating stage or age (Ohwada et al. 2002). An increase of pepsinogen reflects mucousal damage as a consequence of Ostertagia infection (Schaw et al. 1997 and Scott et al. 1999) and abomastitis in cows (Schillhorn, 1988). On the other hand, Ohwada et al. (2002) observed remarkable decreases of serum pepsinogen concentrations in cows with left and right DA and those with abomasal volvulus (AV). These phenomena suggest the pathogenesis of atrophic gastritis. Although their report showed different pepsinogen values in each DA type at operation, the changes after operation were not described. Therefore, it could not be confirmed if the mucosal condition recovered.

The purpose of this study was to carry out an epidemiological investigation in cows with DA and to evaluate the recovery of abomasal condition by determining serum pepsinogen and other biochemical values.

## Materials and Methods

### *Animals*

One hundred twenty-three Holstein cows with DA were used in this study. The animals were fed on 89 farms of the Kushiro region in Hokkaido, Japan from October 2004 through February 2005. Cows with DA were divided into those with left displacement of the abomasum (LDA; n=89,  $4.7 \pm 1.8$  yr), right displacement of the abomasum (RDA; n=20,  $5.0 \pm 2.2$  yr) and abomasal volvulus (AV; n=14,  $3.9 \pm 0.9$  yr). All cows were diagnosed based on physical examination (ping sound and decreased appetite). The diagnosis was additionally verified surgically when an operation took place.

### *Epidemiological investigation*

Occurrence time, culling within 2 months after operation and the stall system were investigated for all farms.

### *Blood Sampling*

Ten milliliters of blood was collected from the jugular vein of each cow with DA into an evacuated sterile tube without any anticoagulant three times (just before operation (day 0), days 1-3 and days 7-11 after operation). Samples were rapidly centrifuged at 3,000 rpm for 15 minutes. Sera were harvested and aliquots were stored at  $-20^{\circ}\text{C}$  until analysis. Moreover, 5 ml of blood was allowed to flow freely from the jugular vein of each cow into a tube containing sodium fluoride as an anticoagulant (Kelly, 1984).

### *Serum and blood biochemical analysis*

Serum concentrations of nonesterified fatty acids (NEFA), chloride and calcium and the blood glucose value were measured at Kishimoto Clinical Laboratory, Sapporo, Japan.

### *Serum pepsinogen determination*

The serum pepsinogen value was measured according to the method of Berghen et al. (1987).

### *Statistical analysis*

The obtained data were compared and analyzed by Scheffe's multiple comparison F test using SAS, 2nd Ed, (Institute Inc. Cary, NC, USA). Moreover the results were tested for significance using the t-test according to Selvin (1996).

## Results

The occurrence rates of LDA, RDA and AV during the first three weeks after parturition were 80.9% (72/89), 65.0% (13/20) and 57.1% (6/14), respectively (Table 1). The percentages of dead and disused animals were higher for AV (28.6%) and LDA (23.6%) than for RDA (5%).

The serum NEFA concentration in all cows with DA at day 0 ( $1,338 \pm 546 \mu\text{Eq/L}$ ) was higher than the reference value. Significant increases of NEFA concentrations in cows with LDA were observed compared to those with AV and RDA.

**Table 1** Occurrence time, culling and stall type in cows with displaced abomasum

Type	No	Occurrence						Culling		Stall	
		*1W	2W	3W	4W	5W	5W<	Died & Culled	Survived	Free stall	Tie stall
LDA	89	32 (36.0)	26 (29.2)	14 (15.7)	6 (6.7)	3 (3.4)	8 (9.0)	21 (23.6)	68 (76.4)	46 (52.7)	43 (48.3)
RDA	20	8 (40.0)	1 (5.0)	4 (20.0)	3 (15.0)	2 (10.0)	2 (10.0)	1 (5.0)	19 (95.0)	10 (50.0)	10 (50.0)
AV	14	2 (14.0)	1 (7.0)	5 (36.0)	1 (7.0)	0 (0.0)	5 (36.0)	4 (28.6)	10 (71.4)	7 (50.0)	7 (50.0)
Total	123	42 (34.1)	28 (22.8)	23 (18.7)	10 (8.1)	5 (4.1)	15 (12.2)	26 (21.1)	97 (78.9)	63 (51.2)	60 (48.8)

LDA: left displacement of the abomasum. RDA: right displacement of the abomasum. AV: abomasal volvulus.

\* Weeks after parturition. Numbers in parenthesis indicate percent.

The serum NEFA concentration in all cows with DA returned to the reference value at 7-11 days. The blood glucose level in cows with LDA was within the normal range, whereas those of cows with RDA and AV were higher than the reference value (hyperglycemia). At 7-11 days after operation, the glucose value returned to the normal range. The majority of cows with DA had hypocalcemia and hypochloremia. Briefly, 6 (42.9%) of the cows with AV, 13 (65.0%) of those with RDA and 35 (39.3%) of those with LDA had calcium concentrations of less than 8 mg/dL (sub-clinical hypocalcemia). There were non-significant variations of calcium and chloride concentrations among cows with LDA, RDA and AV. Calcium and chloride levels were clearly recovered post-operation in all cows with DA, especially in those with LDA. There were non-significant variations of serum and blood biochemical parameters between free stall and tied stall cows with DA.

The pepsinogen values in all cows with DA at day 0 ( $n=123$ ,  $828 \pm 257$  mU) remarkably decreased compared to the reference range, but there were no significant difference among cows with LDA, RDA and AV (Table 2). The values in cows with LDA, RDA and AV at 7-11 days were still lower than the reference value. In addition, the pepsinogen level was lower for dead and disused animals ( $n=26$ ,  $790 \pm 264$  mU) than in healed animals ( $839 \pm 256$  mU).

## Discussion

It has been reported that the occurrence rates of LDA and RDA were within 6 weeks (Radostits et al. 2007) and 3-6 weeks after parturition (Constable et al. 1992 and Delgado-Lecaroz et al. 2000) respectively. However, 80.9% of the cases of LDA and 65.0% of those of RDA happened within 3 weeks postpartum in this study. We could confirm the difference between the present and previous occurrences of DA. This phenomenon may be attributable to the changes of risk factors related to its occurrence. Briefly, it is presumed that cows with DA already fall into NEB before parturition and the close-up period. The pathogenesis induces severe conditions such as shortage of glucose and disturbance of calcium and ketone bodies metabolism (Muylle et al. 1990 and Geishauer, 1995). On the other hand, 42.9% of AV occurred more than 4 weeks after parturition. The direction of DA could be influenced predominantly by the volume of the forestomach. Immediately after parturition, displacement occurs to the left because of a reduction in the size of the rumen volume. Several weeks later the dilated abomasum moves caudally and dorsally in the right abdomen because the volume of the forestomach is much larger, thereby providing an effective barrier (rumen barrier) (Radostits et al. 2007). With regard to the prognoses of different types of DA, the percentage of animals that died or became disused was higher for cows with AV (28.6%) and LDA (23.3%) than for those with RDA

**Table 2** Changes of blood and serum biochemical parameters in cows with displaced abomasum during the experimental period

Type	No	NEFA ( $\mu\text{Eq/L}$ ) (415 $\pm$ 203) <sup>#</sup>			Glucose (mg/dL) (57.6 $\pm$ 8.7) <sup>#*</sup>			Chloride (mEq/L) (105.2 $\pm$ 3.0) <sup>#*</sup>			Calcium (mg/dL) (9.2 $\pm$ 1.3) <sup>#</sup>			Pepsinogen (mU) (900-1200) <sup>***</sup>		
		day 0	days 1-3	days 7-11	day 0	days 1-3	days 7-11	day 0	days 1-3	days 7-11	day 0	days 1-3	days 7-11	day 0	days 1-3	days 7-11
		LDA	89	1435 $\pm$ 549	1081** $\pm$ 426	561** $\pm$ 290	54.4 $\pm$ 20.9	58.4 $\pm$ 14.4	52.4 $\pm$ 9.3	94.2 $\pm$ 6.0	96.5** $\pm$ 6.4	97.9** $\pm$ 3.4	8.1 $\pm$ 0.8	8.4* $\pm$ 0.8	9.2** $\pm$ 0.6	812 $\pm$ 252
RDA	20	1075 $\pm$ 488	882 $\pm$ 402	478** $\pm$ 296	87.5 $\pm$ 60.4	65.2 $\pm$ 18.9	52.7* $\pm$ 6.7	93.0 $\pm$ 9.2	95.7 $\pm$ 7.9	98.9* $\pm$ 4.2	7.7 $\pm$ 1.0	8.2 $\pm$ 1.12	8.8** $\pm$ 0.64	876 $\pm$ 277	961 $\pm$ 460	876 $\pm$ 195
AV	14	1096 $\pm$ 412	845 $\pm$ 449	342** $\pm$ 400	93.9 $\pm$ 33.4	65.3* $\pm$ 18.4	63.1** $\pm$ 16.7	89.2 $\pm$ 10.5	93.5 $\pm$ 6.5	96.4 $\pm$ 13.0	8.2 $\pm$ 0.8	8.2 $\pm$ 1.2	9.3** $\pm$ 0.6	862 $\pm$ 266	776 $\pm$ 185	738 $\pm$ 301

LDA: left displacement of the abomasum. RDA: right displacement of the abomasum. AV: abomasal volvulus.

\* Significant when compared with the value at day 0 (\* $P$ <0.05; \*\* $P$ <0.01).

# Reference value according to <sup>#</sup>Oikawa and Katoh 2002, \*\*Reference value according to Zadnik 2003. \*\*\*Reference value according to Ohwada et al. (2002).

(5.0%). The case fatality rate of AV was consistent with previous reports (23.5%) (Radostits et al. 2007), whereas that of LDA was higher than that previously reported (5.6%) (Constable et al. 1992). This difference may be attributed to the involvement of risk factors related to the occurrence of LDA. Further researches are needed to elucidate this phenomenon.

Regarding the serum biochemical parameters (Table 2), there were remarkable increases of NEFA concentrations in all cows with DA at day 0, compared with the reference value. The serum NEFA concentration in cows with LDA significantly increased compared with those for cows with AV and RDA. This may have been because LDA was strongly associated with NEB. These results were in agreement with the report of Oikawa et al. (1997) that cows with LDA had higher NEFA concentrations. The odds for having LDA were 2 times greater for cows with plasma NEFA >0.3 mEq/L (cut point) between 3-35 days before calving than for cows that had NEFA below the cut point (Geishauser, 1995, Cameron et al. 1998).

In this study hyperglycemia was observed in cows with RDA and AV. Previous authors similarly reported increased blood glucose values in cows with DA (Van Meirhaege et al. 1988, Cupere et al. 1991, Muylle et al. 1990 and Itoh et al. 1998). On the basis of our results we hypothesized that hyperglycemia associated with RDA might be

associated with impaired outflow of pancreatic juice and disturbed blood circulation in the pancreatic parenchyma because of changes of duodenal and omental position due to dislocation of the abomasum (Klucinski et al. 1988, Muylle et al. 1990, Kehrlí and Goff, 1992). Therefore the elevated glucose level observed in RDA might be a secondary finding (Van Winden and Kuiper, 2003 and Van Winden et al. 2003).

The majority of the cows with DA showed subclinical hypocalcaemia (Table 2). Six (42.9%) of the cows with AV, 13 (65.0%) of those with RDA and 35 (39.3%) of those with LDA had calcium levels of less than 8 mg/dL (subclinical hypocalcemia). This pathogenesis is considered to be an important factor to reduce the tone of the abomasal and ruminoreticular walls, which may induce DA. Briefly, previous researchers (Massy et al. 1993, Delgado-Lecaroz et al. 2000 and Radostits et al. 2007) reported that cows with hypocalcemia (serum calcium concentration <7.9 mg/dL) 12 hrs before parturition had a 4-8 times greater risk of developing LDA than did normocalcemic cows. Hypochloremia was more pronounced in cows with AV especially (Table 2). Similar results were reported by Geishauser and Seeh (1996). Moreover experimentally induced reduction of the abomasal emptying into the cow duodenum resulted in hypochloremia after eight hours. The hypothesis is that the ruminant forestomach compartments have the capacity to

absorb  $\text{Na}^+$  and  $\text{Cl}^-$ , and the reflux and sequestration of abomasal secretions that occur in an obstruction/ligation model or naturally occurring obstructions may make these ions, to some degree, not accessible to the plasma by this route (Gable and Martens, 1991 and Ward et al. 1994). There are no published papers about the effect of stall type on biochemical parameters in cows. In this study we confirmed that there were non-significant variations in their parameters between free and tie stall cows with DA, which may indicate that there is no difference of the pathogenesis between the two stall systems for DA.

The pepsinogen value was markedly decreased in all cows with DA ( $n=123$ ,  $828.3 \pm 257.1$  mU) compared with the reference range. The results of this study were generally in agreement with a report that serum pepsinogen concentrations in LDA, RDA and AV cows were remarkably decreased compared with healthy cows (Ohwada et al. 2002). The decreased value of serum pepsinogen in cows with DA is thought to be attributable to atrophy of the mucous membrane of the abomasum. Also in human beings, a decrease of pepsinogen in sera of patients with chronic atrophic gastritis was reported (Samloff et al. 1982, Dinis-Ribeiro et al. 2004 and Weck and Brenner 2006). Pepsinogen values in cows with LDA, RDA and AV at 7-11 days post-operation were still lower than the reference value, although almost biochemical parameters returned to the normal levels after operation. This finding indicates that the mucosa of the abomasum did not recover even at that time.

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## 要 約

本研究は、第四胃変位牛の発生に関する疫学調査を行うとともに、術後の回復状況を血清ペプシノーゲン値およびその他の生化学値を測定することによって評価することを目的に実施された。供試牛として123頭の第四胃変位牛が用いられた。分娩後3週間以内の発生率は、第四胃左方変位(LDA)で80.9%、第四胃右方変位(RDA)で65.0%であった。一方、第四胃捻転(AV)の42.9%が分娩4週以上経過してから発生していた。AVの死産率は28.6%、LDAとRDAではそれぞれ23.6%と5.0%であった。手術時にすべての第四胃変位牛において、非エステル型脂肪酸濃度が著明に上昇した。また、RDAとAV牛は高血糖を示した。低カルシウム血症および低クロール血症がいずれの第四胃変位牛にも認められた。フリーストール飼養とタイストール飼養の間には血液および血清生化学値に有意な差は見られなかった。血清ペプシノーゲン値はすべての第四胃変位牛で明らかに低下した。手術後7~10日後において、ほとんどの生化学値は正常範囲に回復していたが、ペプシノーゲン値は依然正常値よりも低値を示していた。この所見は、第四胃粘膜の状況がまだ回復していないことを示唆するものと考えられた。