

## Serum pepsinogen level and abomasal ulcerations in experimental abomasal displacement in sheep

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**ABSTRACT:** It is believed that serum pepsinogen levels could be useful for diagnosis of abomasal changes in cattle. Diagnosis of abomasal displacement (AD) is made via invasive and non-invasive techniques. None of the extant methods is a reliable indication of mucosal change. The applicability of serum pepsinogen levels for the diagnosis of changes in the mucous membrane of the abomasum in experimentally induced left and right AD in sheep was investigated in fourteen rams. Abomasal fluid samples were taken and the pH was recorded. Twelve sheep underwent induced left and right AD (six for each group). Two sheep underwent exploratory laparotomy alone to assess the effect of surgical stress on the abomasum. Blood samples were taken before surgery, at the 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup> and 11<sup>th</sup> days after surgery and at the time of necropsy and serum pepsinogen levels were measured. After two weeks the animals were slaughtered and abomasal fluid pH and types of abomasal ulcers were recorded. Significant changes in pepsinogen levels in the left displaced abomasums (LDA) group were seen on days 11 and 14 after surgery ( $P < 0.05$ ). Significant changes in pepsinogen levels in the right displaced abomasum (RDA) group were seen on Days 9, 11 and 14 after surgery ( $P < 0.05$ ). There was no association among the types of ulcers and the serum pepsinogen levels in AD cases. The pH increased significantly ( $P < 0.05$ ) after induced AD in both groups. There were no significant changes in serum pepsinogen levels on different days after surgery among ulcerated and non ulcerated cases in both LDA and RDA groups ( $P < 0.05$ ). Serum pepsinogen levels were significantly higher in AD groups. There was no association between the types of ulcers and serum pepsinogen levels in AD cases. It seems that the increase in concentration of serum pepsinogen is a good reflection of the damage to the abomasal mucosa due to AD, as was shown by the earlier increase in levels in the course of displacement in the RDA group.

**Keywords:** right displaced abomasum (RDA); left displaced abomasum (LDA); abomasal displacement (AD); abomasal ulcer; pepsinogen; sheep

Abomasal ulcerations occur in both adult cattle and calves. Indications of the loss of the abomasal epithelium may range from no clinical signs to haemorrhage and subsequent melena, through to peritonitis if the erosive processes penetrate all layers of the abomasum. The prevalence of abomasal mucosal diseases in cows is becoming more frequent with modern intensive production (Palmer and Whitlock, 1984; Katchuk, 1992; Cable et al., 1998; Radostits et al., 2007). Clinical signs are often rather diffuse and non-specific and it would be of considerable help to find an association between abomasal ulcers and various clinical parameters. Diagnoses of abomasal ulcers are based on clinical signs and faecal occult blood tests.

Abdominocentesis is used to confirm diffuse peritonitis in perforative abomasal ulcers (Mesaric et al., 2002). Gastroscopy has become an excellent tool to verify the diagnosis in humans, horses and dogs (Oderda et al., 1988; Sandin et al., 1999). In cattle practice, this diagnostic method cannot be used due to the presence of the forestomach and permanent secretion of abomasal juice (Mesaric, 2005). Some authors believe that the determination of pepsinogen levels in blood could be a useful tool for diagnosis of abomasal changes in cattle (Mesaric et al., 2002). Pepsinogen is a proenzyme, produced by parietal cells of the abomasal mucosa. In humans, serum pepsinogen is elevated in different diseases including gastric and duodenal ulcers (Samloff et

al., 1986). Increased conversion pepsinogen into active pepsin by enhanced acidity of gastric contents can cause ulcers in humans and animals (Vianello et al., 1988; Tanaka et al., 1991). In blood, a certain physiological level of pepsinogen is present. The increase in pepsinogen levels due to various diseases and tumours is statistically significant (Mesaric et al., 2002). Although elevated pepsinogen levels induced by non-parasitic diseases such as abomasitis catarrhalis acuta, abomasal ulcerations and left or right abomasal displacement have not been confirmed (Voros et al., 1984; Zadnik and Mesaric, 1999), there are reports that cows with abomasitis, left and right-sided displacements of the abomasum and ulcers, also have increased serum pepsinogen levels (Mesaric et al., 2000). Displaced abomasum represents a group of pathological events stemming from smooth muscle atony and gas and fluid accumulation following displacement of the abomasum from its normal ventral position on the abdominal floor (Geishausser, 1995). Diagnosis of the disease is based on invasive and non-invasive techniques. The invasive methods include laparotomy and laparoscopy, while non-invasive methods are represented by simultaneous auscultation and percussion, sonography, and rectal exploration (Zadnik and Mesaric, 1999; Radostits et al., 2007). Diagnosis is also supported by the analysis of blood, milk, urine, faeces, etc. However, it should be emphasized that none of these methods is a reliable indication of mucosal changes due to abomasal displacement which significantly affects the course and prognosis of the disease (Zadnik and Mesaric, 1999). In this study, the applicability of serum pepsinogen levels for the diagnosis of changes in the mucous membrane of the abomasum in experimentally induced left and right abomasal displacement in sheep was investigated as a model for cattle.

## MATERIAL AND METHODS

Fourteen apparently healthy Iranian crossbred rams aged 2–2.5 years and weighing 45–50 kg were used. Rams were fed a ration based on hay and barley and water was offered *ad libitum*. All rams were dewormed on two occasions, two weeks apart using albendazole (7.5 mg/kg, orally) and ivermectin (0.2 mg/kg *s.c.*). Animals were kept in standard and clean conditions for four weeks. Analysis of faecal samples revealed no parasitic infections. A faecal occult blood test was carried out to rule out any

GI bleeding in animals. Each animal was submitted to a clinical examination and routine hematological investigation. The proven healthy animals were randomly assigned into two equal groups. Blood samples were taken before surgery from the jugular vein and sera were separated with centrifugation for 10 min at 2400 RPM in a normal manner and frozen at  $-20^{\circ}\text{C}$  until analysis. Samples from the abomasal fluid were taken by paracentesis before induced abomasal displacement (control samples). The abomasal fluid samples were obtained percutaneously caudal to the xiphoid process on the ventral midline (Radostits et al., 2007). Immediately after sampling, the pH was recorded using Merck pH-indicator strips. After an overnight fast, allowing water *ad libitum*, animals were anesthetized with intravenous injection of sodium thiopental (16 mg/kg), with additional quantities given as needed to maintain adequate surgical anesthesia. In Group 1, six sheep underwent induced left abomasal displacement (LDA) surgery in a paracostal region of the left flank. After exploratory laparotomy, the greater curvature of the abomasum was fixed to the underlying abdominal wall muscles in the highest part of left flank (Figure 1). In Group 2, six sheep underwent induced right abomasal displacement (RDA) using the same procedure. In each group, one sheep underwent exploratory laparotomy to assess the effect of surgical stress on probable abomasal ulcer formation. All sheep were taken under intensive control and conditions as before and blood samples were taken at the 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup> and 11<sup>th</sup> days after surgery from the jugular vein and sera were separated and stored. After two weeks, all the animals slaughtered. Blood samples were taken before slaughter and serum pepsinogen levels in all samples were determined using a micro-method (Dorny and Vercruyse, 1998). At necropsy, abomasal fluid samples were taken and the pH was recorded immediately, using Merck pH-indicator strips. The abomasum was opened along the greater curvature and placed in a water bath to wash away food particles. Abomasal mucosa were examined and the lesions and the types of ulcers were recorded (Whitlock, 1980). For histological examination, several tissue samples were collected from each type of lesion and fixed in 10% buffered formalin, embedded in paraffin, cut at 5  $\mu\text{m}$ , and stained with haematoxylin and eosin (H&E). Mixed model analysis of variance was used to compare serum pepsinogen level (on different days) and abomasal fluid pH before and after displacement

Table 1. Serum pepsinogen activity, abomasal pH and the presence and types of ulcerations in experimental LDA ( $n = 6$ ) and control sheep

Sheep No.	Serum pepsinogen activity(IU/l)						Abomasal pH		Abomasal ulceration			
	before sur- gery	1 <sup>st</sup>	3 <sup>rd</sup>	5 <sup>th</sup>	7 <sup>th</sup>	9 <sup>th</sup>	11 <sup>th</sup>	14 <sup>th</sup>	before sur- gery	after surgery	presence	type
1	4.42	4.42	4.42	5.6	5.6	5.6	5.71	6.24	4.3	4.5	+	1
2	5.6	5.6	5.6	5.71	6.24	6.24	8.52	8.52	3.2	3.8	+	1,3
3	1.22	1.22	1.22	1.22	1.22	1.02	3.42	3.42	4.3	4.3	-	-
4	1.02	1.22	1.22	2.42	4.22	4.22	4.42	5.42	4.3	5	+	1
5	3.42	2.42	4.22	4.22	4.42	5.6	6.24	6.24	3.8	5.5	-	-
6	2.42	2.42	3.42	4.42	4.42	5.87	6.24	6.24	4.3	5.5	+	1
Mean $\pm$ SE	$3.01 \pm 1.80^a$	$2.88 \pm 1.77$	$3.35 \pm 1.79$	$3.93 \pm 1.78$	$4.35 \pm 1.72$	$4.75 \pm 1.95$	$5.75 \pm 1.75^b$	$6.01 \pm 1.64^b$	$4.03 \pm 0.45$	$4.76 \pm 0.68$		
Control	1.64	1.64	1.22	1.22	1.22	1.22	1.02	1.02	3.8	4.3	-	-

Types of ulcerations: 1 = erosions and non-perforating ulcers, 2 = bleeding ulcers, 3 = perforated ulcers with local peritonitis  
Different letters show significant difference ( $P < 0.05$ )

Table 2. Serum pepsinogen activity, abomasal pH, the presence and types of ulcerations in experimental RDA ( $n = 6$ ) and control sheep

Sheep No.	Serum pepsinogen activity (IU/l)						Abomasal pH		Abomasal ulceration			
	before sur- gery	1 <sup>st</sup>	3 <sup>rd</sup>	5 <sup>th</sup>	7 <sup>th</sup>	9 <sup>th</sup>	11 <sup>th</sup>	14 <sup>th</sup>	before sur- gery	after sur- gery	presence	type
7	4.42	4.22	4.42	4.42	5.62	5.62	5.71	5.87	4.5	4.3	-	-
8	0.48	0.48	1.22	2.42	3.42	4.22	4.42	4.6	4.3	6	-	-
9	3.42	3.42	4.22	4.22	4.42	5.71	5.87	5.87	3.2	4	+	2
10	4.22	4.22	4.42	5.71	5.6	5.87	5.87	5.87	2.5	4.3	+	1
11	0.9	0.9	1.22	1.22	4.42	5.62	5.62	5.62	3.5	5.5	+	1, 2
12	0.11	0.9	2.42	4.42	4.42	5.62	6.24	5.81	3.8	3.8	+	1, 3
Mean $\pm$ SE	$2.25 \pm 1.97^a$	$2.35 \pm 1.77$	$2.98 \pm 1.56$	$3.73 \pm 1.62$	$4.65 \pm 0.83$	$5.44 \pm 0.60^b$	$5.62 \pm 0.62^b$	$5.60 \pm 0.50^b$	$3.63 \pm 0.73$	$4.65 \pm 0.88$		
Control	4.22	4.22	4.42	4.22	4.42	4.42	4.6	4.6	3.8	4	-	-

Types of ulcerations: 1 = erosions and non-perforating ulcers, 2 = bleeding ulcers, 3 = perforated ulcers with local peritonitis  
Different letters show significant difference ( $P < 0.05$ )

Table 3. Serum pepsinogen activity and abomasal pH in ulcerated and non-ulcerated cases in induced abomasal displacements (LDA + RDA)

	Serum pepsinogen activity (IU/l)								Abomasal pH	
	before surgery	after surgery							before surgery	after surgery
		1 <sup>st</sup>	3 <sup>rd</sup>	5 <sup>th</sup>	7 <sup>th</sup>	9 <sup>th</sup>	11 <sup>th</sup>	14 <sup>th</sup>		
Ulcerated ( <i>n</i> = 8)	2.76 ± 1.96	2.88 ± 1.79	3.36 ± 1.60	4.21 ± 1.63	4.91 ± 0.77	5.59 ± 0.59	6.06 ± 1.07	6.19 ± 0.97	3.63 ± 0.65	4.55 ± 0.70
Non-ulcerated ( <i>n</i> = 4)	2.38 ± 1.84	2.08 ± 1.63	2.77 ± 1.79	3.07 ± 1.52	3.67 ± 1.86	4.11 ± 2.16	4.94 ± 1.27	5.03 ± 1.28	4.22 ± 0.29	5.02 ± 0.86

No significant differences between ulcerated and non-ulcerated cases ( $P > 0.05$ )

in LDA and RDA groups. The difference of each factor (serum pepsinogen levels and abomasal pH), before and after abomasal displacement on different days, in ulcerated and non-ulcerated cases was compared by Student's *t*-test. The significance level for each test was set at 5%. This work was in line with local animal welfare regulations.

## RESULTS

The changes in serum pepsinogen levels, pH of abomasal contents and the types of abomasal ulcers in induced LDA and RDA (treatment groups) are presented in Tables 1 and 2, respectively. No abomasal lesions, significant changes in pepsinogen levels or abomasal pH were found in two sheep which underwent an exploratory laparotomy alone. Abomasal ulcers in treatment groups varied in size from a few millimetres to 1–2 cm. They were mostly either round or oval. The ulcers were covered with

a rough grayish material and contained fibrinous exudates. The margins of the ulcers were thickened and showed various degrees of hyperaemia and oedema. Two of the ulcers, (one LDA and one RDA) were type 3 ulcers (perforated ulcer). The macroscopic and microscopic features of the abomasal ulcers are shown, respectively, in Figures 2 and 3. Gross lesions consisted of ulcers, hyperplasia, and congestion in the fundic area. Microscopically, the abomasal lesions were characterized by demarcated areas of mucosal erosion or ulceration. Significant changes in pepsinogen levels in the LDA group were seen on Days 11 and 14 after surgery ( $P < 0.05$ ). Significant changes in pepsinogen levels in the RDA group were seen on Days 9, 11 and 14 after surgery ( $P < 0.05$ ). There was no association between the types of ulcers and the levels of serum pepsinogen in the RDA and LDA cases. The pH increased significantly ( $P < 0.05$ ) after induced abomasal displacement in both groups (LDA and RDA). There wasn't any significant changes in serum pep-



Figure 1. The procedure for fixing the greater curvature of the abomasum to underlying abdominal wall muscles

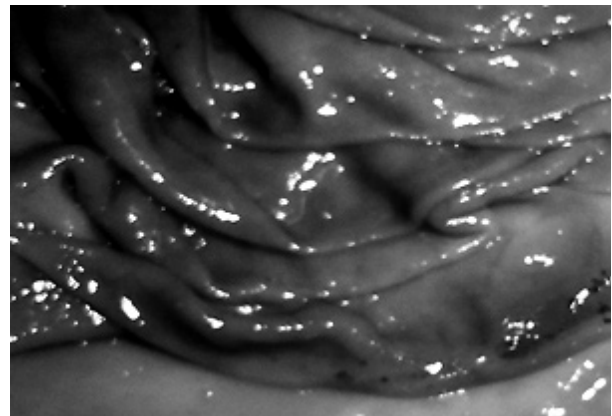


Figure 2. Fundic part of an abomasum showing ulceration, hyperplasia, and congestion



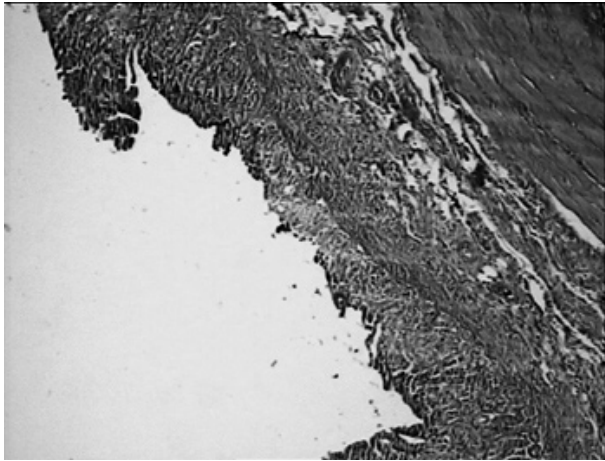


Figure 3. Abomasal tissue showing epithelial erosion and infiltration of mononuclear cells; H&E, 40×

sinogen levels on different days after surgery among ulcerated and non ulcerated cases in both the LDA and RDA groups ( $P < 0.05$ ; Table 3).

## DISCUSSION

In ruminant blood, a certain physiological level of pepsinogen is present (Schillhorn Van Veen, 1988; Mostofa and McKellar, 1990; Andren, 1992) and measurement of blood pepsinogen levels is widely used for assessing gastrointestinal parasitism (McKellar et al., 1990; Berghen et al., 1993; Kolb et al., 1993). Harvey-White et al. (1983), Hilderson et al. (1989) and Paynter (1994) found that serum pepsinogen values of zero to 5.0 IU/l are normal for young and mature cattle and are not associated with any clinically relevant damage to the abomasal mucosa. Paynter (1994) reported that the reference range for the mean pepsinogen level in cattle is 2.54–3.54 IU/l. It has been reported that low or normal levels of serum pepsinogen ( $< 5.0$  IU/l) may be useful as a predictor for low susceptibility to major changes to the mucous membrane of the abomasum (Mesaric, 2005). There are conflicting findings about how serum pepsinogen passes from peptic cells to the blood in cattle and two main theories to explain increasing serum pepsinogen levels. The first involves increased epithelial and vascular permeability allowing pepsinogen to leak into the blood (Jennings et al., 1966; Murray, 1969), and the second involves the direct hypersecretion of pepsinogen into the blood from zymogenic cells in a retrograde direction (McKellar et al., 1986; Fox et

al., 1989). Voros et al. (1984) investigated pepsinogen levels in blood, urine and abomasal fluid and reached the conclusion that increased blood levels are found especially in cows with left displacement. Voros et al. (1984) did not establish any statistically significant differences, though pepsinogen values in the LDA were somewhat higher than in the RDA. In the study of Aukema and Breukink (1974), serum pepsinogen levels of cattle with abomasal ulcers with fatal haemorrhages were abnormally high only in half of the investigated animals. However, no exact investigations were made to determine the role of serum pepsinogen in detecting abomasal ulcers in cattle (Mesaric, 2005). Schillhorn van Veen (1988) determined an increased pepsinogen level in the blood of cows with abomasal leukosis. Paynter (1994) reported that the mean serum pepsinogen level was above the physiological range of 5.0 IU/l that was designated as the normal threshold value for cattle. Serum-pepsinogen levels above 5.0 IU/l in cattle denote a serious injury of the abomasal mucosa (Mesaric et al., 2002). Mesaric (2005) reported that the use of serum pepsinogen levels can provide a simple serum test to diagnose or evaluate cows with subclinical abomasal ulcers. Although our present data confirmed that serum pepsinogen levels are significantly higher in LDA and RDA affected sheep, there was no association between the types of ulcers and pepsinogen levels in RDA and LDA cases. Although there were no significant differences in pepsinogen levels between ulcerated and non ulcerated cases, it seems that there is a marked increase in serum pepsinogen levels in ulcerated cases which may be biologically important. Furthermore, our results showed that an increase in serum pepsinogen levels may be a good indicator of the damage to the abomasal mucosa, as shown by the earlier increase in its level in the course of displacement in the RDA group. The results in induced LDA and RDA in sheep can be extended to cattle and may be of value in the diagnosis, prognosis and monitoring of abomasal status in RDA and LDA cases.

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