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Evaluation of glutaraldehyde test and amount of rumen content chlorine in cases of vagal indigestion (Hoflund syndrome) due to reticuloperitonitis traumatica

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ABSTRACT

This study is published in order to present the rumen content, amount of chlorine, and the results of glutaraldehyde test in cattle clinically diagnosed with vagal indigestion (Hoflund syndrome) due to reticuloperitonitis traumatica (RPT). The working material included an experimental group, and a control group. The experimental group was made up of 52 cows of different breeds admitted to our clinic for examination and diagnosed with vagal indigestion due to RPT, their ages ranging between 2-14. The control group was made up of 10 cows of different breeds determined to be healthy in clinical examinations, their ages ranging between 2-8. In total, there were 62 cows. After all cattle in the two groups were clinically examined, a minimum of 100 ml of rumen content samples were taken using rumen sounder. Rumen content chlorine levels was determined according to the Schales-schales method. In order to carry out the glutaraldehyde test in the 25 animals in the experimental group and all the animals in the control group, blood with EDTA was taken from the v. jugularis of the animals. As for the clinical parameters of the animals in the experimental group and the control group; the difference between the arithmetic means of body temperature (38.90 ± 0.55 and 38.68 ± 0.15 °C, respectively), heart frequency (85.92 ± 19.88 and 72.00 ± 7.26 item/minute, respectively), and rumen movement (2.75 ± 1.99 and 8.70 ± 0.48 item/ 5 minutes, respectively) was at the significance level of $P<0.001$, and the difference between the arithmetic means of respiration frequency (29.11 ± 9.94 and 24.80 ± 1.68 item/minute, respectively) was at the significance level of $P<0.01$. In this study, it was observed that the average value of glutaraldehyde test was (2.44 ± 1.18 minutes) in the experimental group, and it was stated that the difference between the groups was $P<0.001$. In conclusion, it is considered that the detection of rumen content chlorine levels, which is the most important parameter in determining abomasal reflux syndrome cases, might be helpful, especially in the diagnosis of functional gastric stenosis, and that the glutaraldehyde test might be useful in detecting back functional stenosis cases due to RPT.

Key words: Hoflund syndrome, vagal indigestion, reticuloperitonitis traumatica, rumen chlorine, glutaraldehyde

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Introduction

Functional gastric stenosis is a form of indigestion characterized by prevention of food transition caused by the stenosis of pylorus, cardia and ostium-reticulo-omasi due to total or partial paralysis of one or more thick ventral and dorsal branches of N. vagus innerving abomasum and forestomach (DIRKSEN, 1994; GUL, 2006; SLANINA, 1985) . The disease, also known as vagus indigestion, was named after Hoflund, who was the first to investigate the disease in 1940, as Hoflund syndrome (BILAL, 2004; DIRKSEN, 1994; SLANINA, 1985; SMITH, 2002).

It is stated that the most important cause of the disease is reticuloperitonitis traumatica (RPT) (BILAL, 2004; DIRKSEN, 1994; GUL 2006; RADOSTITS et al., 2007; REHAGE et al., 1995). Other causes may include growth of mediastinal and retropharyngeal lymph nodes (after tuberculosis or leucosis), mediastinitis, periesophagitis, esophagus diverticula, pileuritis, periesophageal abscesses and pressure by the baby on the N. vagus in animals in their late phase of pregnancy. Rare causes may include actinomycotic lesions, abomasum leucosis, severe abomasitis, eventration diaphragmatic reticuli, omasum obstipation (BILAL, 2004; DIRKSEN, 1994; GUL, 2006) and liver abscesses (DIRKSEN, 1994; SLANINA, 1985; FUBINI et al., 1985).

It is stated generally that it takes different forms such as fore functional (having rumen and reticulum atony, rumen and reticulum active or hyperactivity) and back functional (stenosis of pylorus with or without reticulum atony or recurring functional stenosis of pylorus) gastric stenosis (AYTUG et al., 1991; BILAL, 2004; SMITH, 2002). Cardia stenosis is also regarded as a special form of fore functional stenosis. Though there are small differences between the forms of functional gastric stenosis, the characteristic symptoms are usually the same (DIRKSEN, 1994; GUL, 2006).

Rumen dilatation and nutritional fullness, recurring timpani, gradual weakening and bradycardic symptoms are sufficient to diagnose Hoflund syndrome. Additionally, in back functional stenosis, there is abomasum dilatation and fullness. The disease is long lasting (DIRKSEN, 1994; RADOSTITS et al., 2007).

Clinically it is impossible to differentiate between fore and back functional stenosis. In many cases, it is impossible to determine the cause. In suspicious cases, experimental laparorumenotomy is carried out for precise diagnosis and in order to understand whether it is fore or back functional gastric stenosis (DIRKSEN, 1994; RADOSTITS et al., 2007).

This study is published in order to state rumen content, amount of chlorine, and the results of the glutaraldehyde test in cattles clinically diagnosed with vagal indigestion (Hoflund syndrome) due to RPT.

Materials and methods

The working materials included an experimental group, and a control group. The experimental group was made up of 52 cows of different breeds admitted to our clinic for examination and treatment between 29.02.1996 - 11.04.2006 and diagnosed with vagal indigestion due to RPT, their ages ranging between 2-14. The control group was made up of 10 cows of different breeds supplied from the Ulukent district of the city of Elazığ and determined to be healthy in clinical examinations, their ages ranging between 2-8. In total, there were 62 cows.

Among the cattles in the experimental group, there were 13 Swiss Brown, 10 Holstein, 7 Simmentals, 3 indigenous breeds, 1 Jersey and 18 cross breed.

After all cattle in the two groups were clinically examined (ROSENBERGER, 1990), a minimum of 100 ml of rumen content samples were taken using a rumen sounder. The samples were first examined in terms of their color, smell and density, then their pH was measured using pH papers. Infusoria count was determined using a microscope. When observed with a microscope having a 10x10 objective; a density of infusorias was symbolized as (+++), a medium number of them as (++), few infusorias as (+), and if there was no infusoria, the (-) symbol was used (ROSENBERGER, 1990).

Rumen content chlorine levels were determined according to the Schales-Schales method.

In 5 animals whose heart frequency was below 60, the atropine experiment was carried out as suggested by DIRKSEN and RANTZE (1968). Heart frequency was counted before injection. Then, 40 mg of Atropine sulfate (1% solution) was applied to the neck subcutaneously. 15 minutes later, heart frequency was counted again, and a minimum of 15.8% of increase in heart frequency was regarded as vagus bradycardia.

In order to carry out the glutaraldehyde test in the 25 animals in the experimental group and all the animals in the control group as stated by researchers (ASLAN and OK, 1991; BRAUN et al., 1989; TURGUT, 2000), blood with EDTA was taken from v. jugularis of the animals. The blood samples were centrifuged in 2500 cycles, later thier plasmas were separated and 2 ml of plasma was taken in a 10 ml of plastic injector. 2 ml of glutaraldehyde solution was added and the mixture was checked every 30 seconds in order to see whether coagulation had occurred or not. The evaluation of the test is as follows:

If coagulation occurs in 0-5 minutes, there is severe inflammation

If coagulation occurs in 6-10 minutes, there is medium inflammation

If coagulation occurs in 11-15 minutes, there is little inflammation

If coagulation occurs in more than 15 minutes, the test is negative (there is no inflammation).

In order to determine the statistical differences between the experimental group and the control group, arithmetic means and standard deviations of the recorded data, and the degree of importance of the difference between the groups were defined according to the t-test using SSPS MS Windows Release 12.0.

Results

The arithmetic means, minimum-maximum values of the general clinical examination findings (body temperature, heart and respiration frequency, and the number of rumen movements) of the animals in the experimental group and the control group, and the statistical importance of the differences between the groups are shown in Table 1, whereas the arithmetic means, minimum-maximum values of laboratory examination findings (rumen content chlorine levels and glutaraldehyde test) and the statistical importance of the differences between the groups are shown in Table 2.

Table 1. The arithmetic means, minimum-maximum values of the general clinical examination findings (body temperature, heart and respiration frequency, and the number of rumen movements) of the animals in the experimental group and the control group, and the statistical importance of the differences between the groups

	Control group (X ± Sx)	Experimental group (X ± Sx)	t value	Level of importance
Body temperature (^{0}C)	38.68 ± 0.15 38.5 - 39.0	38.90 ± 0.55 38.0 - 40.3	9.253	**
Heart frequency (item/min)	72.00 ± 7.26 68 - 76	85.92 ± 19.88 44 - 132	8.943	**
Respiration frequency (item/min)	24.80 ± 1.68 24 - 28	29.11 ± 9.94 16 - 68	5.128	*
Rumen movements (item /5 min)	8.70 ± 0.48 8 - 9	2.75 ± 1.99 0 - 6	14.118	**

* P<0.01; ** P<0.001

According to the anamnesis, the cattle in the experimental group did not have any appetite, they were weakening, the amount of milk they produced was decreasing and they sometimes became swollen. In the clinical examinations it was determined that they did not have any appetite, they were weak, there was especially rumen fullness and dilatation, the right ventral abdominal wall had expanded and was suspending against the ventral (looking like an apple or a pear), and it was found that in many of the patients the hair was lustreless, hard and matted, the skin had lost its elasticity, they produced very little faeces, and it was black. All of the patients could stand, however they stood still

and did not want to move. In the rectal examination, it was found out that the rumen was full and had expanded. As for the physical features of the rumen liquid of the animals in the experimental group, the pH value was found to be between 5.5-7, the color varied from green brown to yellow brown depending on whether the animal was in a stall or in a meadow, most of them were stinky, in some patients viscosity had increased and it was foamy, and there was a significant decrease (in + level) in the number of infusoria in all cases. The diseased animals gave positive results to ache experiments in various degrees, and the ferrescope examinations were also positive. Bradycardia was defined in five animals, whereas tachycardia was defined in 30 of them. In animals having bradycardia, an atropine experiment was carried out, and it was found out that in the end heart frequncies increased by more than 16%.

Table 2. The arithmetic means, minimum-maximum values of laboratory examination findings (rumen content chlorine amount and glutaraldehyde test) of the animals in the experimental group and the control group, and the statistical importance of the differences between the groups

	Control group (X ± Sx)	Experimental group (X ± Sx)	t value	Level of importance
Rumen content chlorine amount (mEq/L)	19.50 ± 5.19 14 - 29	55.64 ± 27.51 37 - 171	8.529	**
Glutaraldehyde test (min)	Negative (above of 15 minute)	2.44 ± 1.18 1 - 5	29.001	**

** P<0.001

Discussion and conclusion

The clinically observed findings in the animals in the experimental group such as lack of appetite, weakening, the apple or pear-like appearance of the abdomen when seen from the back, rumen timpani, rumen fullness and dilatation, and the reduction in faeces are in accordance with the literature (AKSOY, 1981; DIRKSEN, 1994; KUIPER and BREUKINK, 1986a and 1986b; STATTLER et al., 2000) stating that these findings may be observed in functional gastric stenosis.

As can be seen from Table 1, as for the clinical parameters of the animals in the experimental group and the control group; the difference between the arithmetic means of body temperature (38.90 ± 0.55 and 38.68 ± 0.15 °C, respectively), heart frequency (85.92 ± 19.88 and 72.00 ± 7.26 item/minute, respectively), and rumen movement (2.75 ± 1.99 and 8.70 ± 0.48 item/ 5 minutes, respectively) is at the significance level of P<0.001, and the difference between the arithmetic means of respiration frequency (29.11 ± 9.94 and 24.80 ± 1.68 item/minute, respectively) is at the significance level of P<0.01.

Atropine sulfate was injected into 5 cows with bradycardia. 15 minutes later, the heart frequency increased up to a minimum of 15.8%. This shows that there is permanent vagus injury with 95% reliability, as stated by DIRKSEN and RANTZE (1968). The number of patients with bradycardia was few. This may be because tachycardia occurred due to intoxication (DIRKSEN, 1994; GUL, 2006) which developed as a result of deterioration of rumen content because of the late arrival of the patients to our clinic. Occurrence of tachycardia in the patients supports the idea that bradycardia is a very important finding, however it is never a specific symptom of vagus lesions.

Rumen content in the animals in the experimental group was stinky, partially foamy, its activity had decreased, and the number of infusoria was reduced. These facts are in compliance with the sources (AYTUG et al., 1991; BRAUN et al., 1990a; DIRKSEN, 1994; KUIPER and BREUKINK, 1986b).

In ruminants, the abomasum content (containing HCl acid) goes back in the reverse direction to the forestomach. This situation may be named as the Abomasal Reflux Syndrome. It is stated in the sources (AKSOY, 1981 and 1983; ANDERSON, 1980; DIRKSEN, 1994) that among the most common causes of abomasum reflux syndrome are: abomasum diseases (the abomasum changes its place or turns, inflammation and ulcers, nutritional fullness, leucosis, gathering of sand in abomasum), mechanical pylorus stenosis, gastric-intestinal atonys, local or common abdominal membrane inflammations, ileus in small intestines, expanding and turning of the blind intestine, the pressure of the baby on the abomasum in late phases of pregnancy, phlegmone inflammations of the omentum near the pylorus, and functional gastric stenosis. It is considered that, in the animals in the experimental group, abomasal reflux began to occur as a result of vagal indigestion due to abscesses and adhesions caused by RPT (GUL, 2006; SLANINA, 1985).

In the diagnosis of abomasal reflux syndrome, the only accepted parameter is the determination of chlorine concentration in the rumen content (AKSOY, 1981; ANDERSON, 1980; BRAUN et al., 1990a and 1990b; BREUKINK and KUIPER, 1980). An increase (above 30 mEq/L) in the amount of chlorine in the rumen content is regarded as a sign of reflux by the abomasum to the forestomach (ELIZONDO, 1975). This idea is supported by the fact that in all the animals in the experimental group, the amount of chlorine was above 30 mEq/L (55.64 ± 27.51 mEq/L), and when compared to the animals in the control group (19.50 ± 5.19 mEq/L), the significance level of the difference between the arithmetic means is as high as $P<0.001$.

ELITOK (1999) found out that the amount of chlorin in rumen content in cattle having vagal indigestion was on average 48.10 ± 6.76 mEq/L. BRAUN et al. (1990b) determined this number to be on average 47.30 ± 4.51 mEq/L in cattle diagnosed with back functional pylorus stenosis. The rumen content chlorine values defined in our patients (on average

55.64 mEq/L) comply with the literature (BRAUN et al., 1990b; ELITOK, 1999). This fact shows that the cases had back functional stenosis.

Various researchers (BRAUN et al., 1990a; KUIPER and BREUKINK, 1986b) stated that chlorine levels increased in rumen content in cattle having fore functional gastric stenosis due to reticulo-omasal stenosis. In the literature (KUIPER and BREUKINK, 1986b) it is stated that clinically it is not possible to differentiate between fore and back functional stenosis, however; because chlorine concentration increases in rumen content in back functional stenosis, and abomasal reflux syndrome, and hypochloremic and hypokalemic alcalosis develop, these two stenoses may be differentiated. The high chlorine levels in the rumen content of the animals in the experimental group complies with these ideas. Thus, the idea that the cases have back functional stenosis is supported.

The pH of rumen content decreases not only in "lactate acidosis (or acidosis due to milk acid)" events, but also with the returning of abosum content containing hydrochloric acid back to the forestomach (HCl acidosis) (ELIZONDO, 1975). However there was no significant decrease in the pH value of the rumen content of some of the cattles in the experimental group. This may lead to the idea that acidic abomasum content (pH: 2.0-4.5) is balanced with the bumper capacity of the forestomach. However, we are of the opinion that possibly saliva involvement due to receiving rumen content with the sounder was also effective.

The existence of RPT in the animals in the experimental group is in accordance with the sources (ANDERSON, 1980; DIRKSEN, 1994; GUL, 2006; RADOSTITS et al., 2007; SLANINA, 1985) stating that the most important cause of functional stenosis is RPT.

It is stated in the literature (BRAUN et al., 1990a, DIRKSEN, 1994; GUL, 2006; OK and ASLAN, 1994; OZBA et al., 1996) that along with classical methods, such as clinical symptoms and ache experiments, biochemical examinations, radiography, and glutaraldehyde tests are also used in the diagnosis of RPT. Fibrinogens and immunoglobulins are semiquantitatively measured with the glutaraldehyde test (ASLAN et al., 1993; ASLAN and OK, 1991). In the sources it is stated (TURGUT, 2000) that glutaraldehyde test gives important clues in the diagnosis and prognosis of diseases characterized by inflammation in cattle, and coagulation occurs within 1-5 minutes in the glutaraldehyde test in cattles with RPT and pericarditis traumatica. A study carried out by OZBA et al. (1996), detected the value of glutaraldehyde test in acute RPT cases as 2.71 ± 0.43 minutes, in chronic RPT cases as 1.71 ± 0.45 minutes, and in the control group as negative. BRAUN et al. (1990a) detected the glutaraldehyde test in 20 cattles diagnosed with vagal indigestion due to omasal transport inefficiency as 5.9 ± 3.04 minutes, and stated that a shortened glutaraldehyde test finding is typical for chronic inflammations. In this study, it was observed that the average value of the glutaraldehyde test (2.44 ± 1.18 minutes) is equal

to those regarded as severe inflammation in the literature (TURGUT, 2000), and it was stated that the difference between the groups was in $P<0.001$ level.

In the sources (AKSOY, 1981; BREUKINK and KUIPER, 1976; ELIZONDO, 1975; KUIPER, 1980), it is stated that prognosis should always be regarded as suspicious in Hoflund syndrome cases. We are of the opinion that a severe inflammation and autointoxication might effect prognosis in animals on whom glutaraldehyde test has been carried out.

In conclusion, it is considered that the detection of rumen content chlorine levels, which is the most important parameter in determining abomasal reflux syndrome cases, might be helpful especially in the diagnosis of functional gastric stenosis, and that the glutaraldehyde test might be useful in detecting back functional stenosis cases due to RPT.

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SAŽETAK

Istraživanje je provedeno s ciljem da se odredi sadržaj buraga, količina klora i rezultati glutaraldehidnog testa u goveda s dijagnosticiranom vagusnom indigestijom (Hoflundovim sindromom) uzrokovanim traumatskim retikuloperitonitisom. Istraživanje je provedeno na pokusnoj i kontrolnoj skupini goveda. Pokusna skupina sastojala se od 52 krave u dobi od 2 do 14 godina, različitih pasmina, koje su bile pregledane na klinici, a dijagnosticirana im je vagusna indigestija zbog retikuloperitonitisa. Kontrolna skupina sastojala se od 10 krava različitih pasmina, u dobi od dvije do osam godina, zdravih prilikom kliničke pretrage. Ukupno su obrađene 62 krave. Nakon kliničke pretrage, sondiranjem je uzeto najmanje 10 mL buražnoga sadržaja. Količina klora u buražnom sadržaju određena je po Schales-schales metodi. Za određivanje glutaraldehida u 25 krava pokusne skupine i svih krava kontrolne skupine krv s EDTA bila je uzeta iz jugularne vene. S obzirom na klinički nalaz ustanovljene su razlike između pokusne i kontrolne skupine. Ustanovljena je razlika ($P<0,001$) u aritmetičkoj sredini tjelesne temperature između pokusne skupine (38,90 ± 0,55 °C) i kontrolne skupine (38,68 °C ± 0,15 °C), frekvenciji bila, koja je za pokusnu skupinu iznosila 85,92 ± 19,88, a za kontrolnu 72,00 ± 7,26 i broju buražnih kontrakcija koji je za pokusnu skupinu iznosio 2,75 ± 1,99, a za kontrolnu 8,70 ± 0,48 u pet minuta. Aritmetička sredina frekvencije disanja za pokusnu skupinu bila je 29,11 ± 9,94, a za kontrolnu skupinu 24,80 ±1,68 u minuti ($P<0,01$). Prosječna vrijednost glutaraldehidnog testa u pokusnoj skupini iznosila je 2.44 ± 1.18 u minuti. Razlika među skupinama bila je na razini $P<0,001$. Zaključuje se da dokazivanje količine klora u buražnom sadržaju kao najvažnijega pokazatelja za određivanje sindroma abomazalnog refluksa može biti od posebne koristi za dijagnostiku funkcionalne želučane stenoze. Glutaraldehidni test može biti od koristi za određivanje u slučajevima povratne funkcionalne stenoze zbog traumatskog retikuloperitonitisa.

Ključne riječi: Hoflundov sindrom, vagusna indigestija, traumatski retikuloperitonitis, burag, klor, glutaraldehid