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Evaluation of Serum Proteins in Water Buffaloes (*Bubalus bubalis*) with Abomasal Ulcer

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ABSTRACT

High prevalence of occurrence of abomasal ulcers has been reported in slaughtered water buffaloes. To evaluate the serum protein electrophoresis in water buffaloes with abomasal ulcers, the abomasums of 100 randomly selected water buffaloes were examined after slaughter. Electrophoresis separation of serum proteins was carried out using cellulose acetate strips. Type I abomasal ulcers were found in 56 buffaloes. The affected buffaloes had significantly lesser total globulin ($p = 0.004$) and γ-globulin ($p = 0.038$) and a higher albumin percent ($p<0.001$) and albumin to globulin ratio ($p<0.001$) in comparison to healthy water buffaloes. The affected buffaloes also had marginally significant lesser serum total protein ($p = 0.068$). Comparison of the measured serum factors between buffaloes with different ulcer locations in the abomasum revealed no significant difference. Separate evaluation of both sexes and different age groups of buffaloes showed no major difference in the results. Age revealed significant correlations with the serum concentration of γ-globuhn ($r = -0.2$, $p = 0.05$), albumin/globulin ratio ($r = 0.24$, $p = 0.018$), albumin percent ($r = 0.23$, $p = 0.024$) and total globulin percent ($r = -0.229$, $p = 0.025$). Age also had marginally significant correlations with α-globulin percent ($r = 0.187$, $p = 0.069$) and γ-globulin percent ($r = -0.188$, $p = 0.066$). Although more work on a larger number of animals is required, it seems that the serum protein electrophoresis may be useful in diagnosis of type I abomasal ulcers in water buffalo.

Key words: Serum proteins, electrophoresis, abomasal ulcer, diagnosis, *Bubalus bubalis*

INTRODUCTION

There are numerous reports regarding the occurrence and prevalence of gastric ulcers in humans, cattle and swine. Different age groups of ruminants may be affected by abomasal ulcers (Braun *et al*., 1991). Whitlock (1980) divided abomasal ulcers into four types, each type producing distinct clinical signs. However, Type I ulcers, non-perforating erosions of abomasal mucosa, have mild or undetectable clinical signs and are usually not diagnosed until slaughter. Braun *et al*. (1991) reported that 20.5% of slaughtered cows had type I abomasal ulcer. According to the results of Aukema and Breukink (1974) study, the prevalence of abomasal ulcers or ulcer scars was 6.3% of slaughtered cows. Although, the aetiology and pathogenesis of abomasal ulcers are not completely known, the different prevalence rates of abomasal ulcers reported in different studies. Clinical status of animals, nutrition, concurrent diseases and stress factors have been proposed as the probable factors causing the different observed prevalence rates (Braun *et al*., 1991; Dirksen, 1994; Ghadrdan-Mashhadi *et al*., 2010). Braun *et al*. (1991) believed that clinically normal animals mainly have type I ulcers, whereas animals with clinical signs of disease often have type 2 to 4 ulcers.

The economic effect of abomasal erosions in ruminants has not yet been investigated, however, physical discomfort resulting in reduced feed consumption and weight gain have been proposed as the probable causes of loss (Jensen *et al*., 1992).

Iranian water buffalo (*Bubalus bubalis*) has a high economic value by providing meat, milk and labor for local farmers. Ghadrdan-Mashhadi *et al*. (2010) reported a relatively high prevalence of occurrence of Type I abomasal ulcers (63.5%) in slaughtered water buffaloes in Iran. Despite the probable importance of abomasal ulcers in water buffalo in Iran, there is little information about the clinical signs of abomasal ulcer in water buffalo and to the best of our knowledge, there has been no previous study regarding the diagnostic aspects of abomasal ulcers in this species.

Alterations in serum protein concentrations are among the most common biochemical markers measured routinely as screening tests for detecting underlying disease or for monitoring disease activity and serum protein levels are the most important laboratory abnormality in a small number of diseases (Nazifi *et al*., 2009; Putignano *et al*., 2000). Changes in serum proteins may not be specific but they are helpful in the diagnosis of abomasal ulcers in water buffalo, however, to the best of our knowledge there has been no previous study regarding the changes of the serum protein electrophoresis in abomasal ulcers in water buffalo and this study was undertaken to compare the serum proteins between affected and non-affected water buffalo.

MATERIALS AND METHODS

Animals and sampling: The investigation was carried out on water buffaloes (*Bulbalus bubalis*) which were slaughtered in a slaughter house reserved only for buffaloes in Ahvaz city, Southwestern Iran, from December 2009 to January 2010 and April to May 2010.

After clinical examination and before slaughter, jugular blood samples in plane tubes, free from anticoagulant, were collected from 100 clinically healthy water buffaloes. Immediately after slaughter, the abomasums of the animals were opened along the greater curvature and washed in water to free ingesta. The abomasal mucosa was examined and the type, number and location of lesions were recorded. The most frequent type of lesion was referred to as the main lesion (Braun *et al*., 1991). Tissue samples were collected from the lesions for histological examination. Buffaloes were of both sexes with different ages and were selected randomly. The age of the animals was estimated using dental characteristics. All animals had grazed the previous summer on ranges around the city. The blood serum was separated after centrifugation at 1800 g for 10 min and the serum samples stored at -20°C until analysis.

Assays: Serum total protein was measured by Biuret method (Latimer *et al*., 2003). Electrophoresis separation of serum proteins was carried out using cellulose acetate strips (voltage of 180 V and about 15 mA for 15 min, apparatus: Elphor, Germany). The strips were subsequently evaluated using densitometery.

Statistical analysis: Statistical analysis was performed using SPSS12 (Illinois, Chicago). The correlation of serum proteins with age and with the number of abomasal ulcers was analyzed by Pearson's correlation tests. Two sample t-tests were used to compare the two sexes and affected and non-affected buffaloes. Analysis of variance (ANOVA) tests were used to compare the serum proteins between buffaloes with different ulcer locations in the abomasum. Differences were considered significant at $p<0.05$.

RESULTS

Overall, 67 male buffaloes and 30 female buffaloes were sampled. The average ages (Mean±SEM) of the male and female buffaloes were 2.19±0.1 and 2.68±0.3 years, respectively. There was no significant difference between the two sexes in the age and measured serum parameters ($p>0.05$).

The results of the measured serum proteins in both sexes, in affected and non-affected buffaloes and in different age groups are shown in Table 1.

Fifty buffaloes were examined in each sampling period and 43 and 20 buffaloes were diagnosed as affected in the first and second sampling periods, respectively. All abomasal lesions were classified as type I abomasal ulcers and were confirmed in histopathological examination. There were significant differences between affected and non-affected buffaloes in the serum concentrations of total globulin ($p = 0.004$), γ-globulin ($p = 0.038$), Albumin/Globulin (A/G) ratio ($p<0.001$) and in albumin and total globulin percents (both $p<0.001$). Serum total protein also had a marginally significant difference between affected and non-affected buffaloes ($p = 0.068$). Serum γ-globulin values had the most significant numerical difference between affected and non-affected buffaloes. There was no significant difference between the affected and non-affected buffaloes in age (2.47±0.16 and 2.06±0.16, respectively). The number of abomasal ulcers had a significant correlation with α-globulin percent ($r = 0.222$, $p = 0.029$) and had a marginally significant correlation with the γ-globulin concentration ($r = -0.19$, $p = 0.063$). Comparison of the measured serum factors between buffaloes with different ulcer locations in the abomasum consisting of pylorus, fundus and glandular regions, revealed no significant difference.

The age showed significant correlations with the serum concentration of the γ-globulin ($r = -0.2$, $p = 0.05$), A/G ratio ($r = 0.24$, $p = 0.018$) and with albumin and total globulin percents ($r = 0.23$, $p = 0.024$ and $r = -0.229$, $p = 0.025$, respectively). Age also had marginally significant correlations with the α-globulin percent ($r = 0.187$, $p = 0.069$) and γ-globuhn percent ($r = -0.188$, $p = 0.066$). Both sexes were evaluated separately, however, the results of the comparison of the measured serum factors between the affected and non-affected buffaloes showed no difference. The buffaloes were

Table 1: The concentrations (Mean±SEM) of serum proteins in affected and non-affected buffaloes and in different genders and age groups

						Age groups		
Parameters	All sampled buffaloes	Affected buffaloes	Non-affected buffaloes	Male buffaloes	Female buffaloes	G1 (≤2 years)	G2 (2 years< and ≤5 years)	G3 (5 years<)
No. of buffaloes	100	63	37	69	31	64	33	3
Total protein (g dL^{-1})**	7.16±0.06	7.08±0.076	7.32±0.105	7.15±0.073	7.18±0.12	7.21±0.08	7.09±0.1	6.98±0.29
Albumin (g dL^{-1})	3.1±0.028	3.1±0.035	3.1±0.046	3.09±0.03	3.13±0.06	3.1±0.035	3.1±0.046	3.2±0.18
Albumin (%)*	43.3±0.002	43.9±0.2	42.4±0.300	43.2±0.2	43.6±0.4	43.2±0.2	43.2±0.3	46.05±1.2
Total globulin (g dL^{-1}) *	4.06±0.041	3.97±0.05	4.22±0.07	4.06±0.05	4.05±0.08	4.09±0.05	4.028±0.07	3.76±0.15
Total globulin (%)*	56.6±0.002	56.1±0.2	57.6±0.300	56.7±0.2	56.4±0.4	56.7±0.2	56.7±0.3	53.9±1.1
α-globulin (g dL^{-1})	1.2±0.014	1.19±0.016	1.22±0.025	1.19±0.016	1.22±0.027	1.2±0.018	1.21±0.024	1.2±0.017
α-globulin (%)	16.75±0.001	16.8±0.1	16.7±0.200	16.65±0.2	16.97±0.2	16.6±0.1	17.01±0.2	17.2±0.4
β-globulin (g dL^{-1})	1.04±0.023	1.01±0.03	1.09±0.04	1.06±0.03	0.99±.04	1.04±0.03	1.02±0.036	1.05±0.17
β-globulin (%)	15.5±0.003	14.25±0.3	14.9±0.400	14.8±0.3	13.7±0.5	14.47±0.3	14.5±0.4	15±2.1
γ-globulin (g dL^{-1}) *	1.82±0.03	1.78±0.03	1.9±0.05	1.81±0.03	1.85±0.056	1.85±0.03	1.79±0.05	1.51±0.04
γ-globulin (%)	25.4±0.003	25.1±0.3	26±0.400	25.3±0.3	25.7±0.6	25.7±0.3	0.25.2±0.4	21.7±1.2
A/G ratio*	0.767±0.006	0.78±0.7	0.74±0.01	0.764±0.01	0.775±0.014	0.76±0.01	0.76±0.011	0.85±0.04

*The difference between affected and non-affected buffaloes is significant ($p<0.05$). **The difference between affected and non-affected buffaloes is marginally significant ($p = 0.068$)

divided into three groups, according to their age as $G_1 \leq 2$ years, 2 years$<G_2 = 5$ years and, $G_3 > 5$ years. Different age groups of buffaloes were also evaluated separately and the results of the comparison of the measured serum factors between the affected and non-affected buffaloes showed no difference, nor were the differences between the affected and non-affected buffaloes in the serum γ-globulin and total globulin percent in the G1 group significant.

DISCUSSION

Although serum proteins electrophoresis in water buffalo and their changes due to some diseases have been investigated in previous studies (Saleh *et al*., 2008; Borghese, 2005; Khadjeh and Razi-Jalali, 2003), to the best of our knowledge, there has been no previous research regarding the changes of serum proteins electrophoresis due to abomasal ulcers in water buffalo.

In the current study, four major classes of proteins, consisting of albumin, α-globulin, β-globulin and γ-globulin and the γ-globulin as the main globulin, were detected in water buffalo. The observed serum protein concentrations and the electrophoretic pattern in healthy buffaloes in our study were basically similar to the previous reports (Saleh *et al*., 2008; Khadjeh and Razi-Jalali, 2003); however, there were some differences. Saleh *et al*. (2008) evaluated the serum protein electrophoretic pattern in water buffalo in Egypt. The serum total protein and the electrophoretic pattern in healthy buffaloes in the current study were similar to their reported ranges for healthy buffaloes. Khadjeh and Razi-Jalali (2003) assessed the serum protein concentration and the electrophoretic pattern in Iranian healthy buffaloes. In comparison to our results, Khadjeh and Razi-Jalali, (2003) reported similar serum total protein and α-globulin, higher albumin and A/G ratio and lesser total globulin, β-globulin and γ-globulin in Iranian water buffalo (Table 2).

According to present results, age had positive correlations with serum albumin percent, A/G ratio and α-globulin percent and negative correlations with serum γ-globulin and total globulin percent. Khadjeh and Razi-Jalali (2003) found that age had a negative correlation with the albumin but had positive correlations with the total globulin and α-globulin which was in contrast to our findings. The effect of some factors such as age, sex, breed, pregnancy, season, geographic and dietary factors on the serum proteins may cause the observed differences (Khadjeh and Razi-Jalali, 2003; Mostaghni *et al*., 1996).

Change in the blood serum proteins following tissue destruction and inflammation is expected (Saleh *et al*., 2008). It has been shown that during inflammation, the synthesis of positive acute phase proteins in liver increases and, concomitantly, the synthesis of negative acute phase proteins, such as albumin, decreases. Blood albumin concentration falls gradually during infectious and inflammatory diseases (Eckersall, 2008; Saleh *et al*., 2008). Despite hypoalbuminaemia which

Table 2: The concentrations of serum total protein and electrophoretic pattern in healthy water buffaloes in the current study and previous studies

Parameters	Current study (Iranian water buffalo)	Saleh *et al*. (2008) (Egyptian water buffalo) Observed range)	Khadjeh and Razi-Jalali (2003) (Iranian water buffalo)
No. of buffaloes	37	25	154
Total protein (g dL^{-1})	7.32±0.105	7.04±0.11 (5.82-7.97)	7.37±0.62
Albumin (g dL^{-1})	3.1±0.046	3.25±0.05 (2.74-3.81)	4.02±0.75
Total globulin (g dL^{-1})	4.22±0.07	3.79±0.08 (2.85-4.63)	3.43±0.62
α-globulin (g dL^{-1})	1.22±0.025	0.977±0.04 (0.64-1.37)	1.22±0.037
β-globulin (g dL^{-1})	1.09±0.04	0.782±0.03 (0.64-1.21)	0.76±0.25
γ-globulin (g dL^{-1})	1.9±0.05	2.03±0.06 (1.56-2.6)	1.35±0.62
A/G ratio	0.74±0.01	0.857±0.018 (0.72-1)	1.29±0.5

Values are as Mean±SEM

occurs during inflammatory diseases in water buffaloes, increased total serum protein concentration as a result of hyperglobulinaemia has been reported by Saleh *et al.* (2008). According to our results, affected buffaloes had lesser serum total protein, total globulin and γ-globulin and had a higher albumin percent and A/G ratio in comparison to healthy water buffaloes. All detected abomasal ulcers were classified as type I, non-perforating erosions of abomasal mucosa which are mucosal defects and do not penetrate the deeper layers of the abomasum (Smith *et al.*, 1983). In contrast to our results, Aukema and Breukink (1974) found that in hemorrhagic abomasal ulcers (type II) in cattle, the serum protein level has decreased and the serum proteins electrophoresis had no abnormality. It seems that different affected abomasal layers and distinctive consequences of the different types of abomasal ulcers such as severe bleeding in type II ulcers and local peritonitis in type III abomasal ulcers make their effects on the serum proteins different and incomparable. On the other hand, similar to differences in the serum proteins concentrations and distribution between different species and breeds (Abdo *et al.*, 1987), changes in the serum proteins in similar situations may be different.

The decreased total serum globulin concentration of the affected buffaloes is a consequence of the decrease in α, β and γ-globulin and is in contrast to the expected pattern in inflammatory conditions. Blood plasma can leak through the abomasal mucosal defect. It is believed that back diffusion of H^+ ions causes the release of histamine from the local mast cells and consequently further leakage due to microcirculatory of the mucosa (Aukema and Breukink, 1974). Loss of blood albumin and globulin can be recompensed by an increase in their production. Serum albumin is synthesized and secreted by the hepatocytes which is controlled by blood colloid osmotic pressure. Albumin is responsible for 80% of the blood colloid osmotic pressure. Some globulins are produced in the liver, while others are made by the immune system (Eckersall, 2008). Faster response to decrement and a higher replacement rate of the albumin in comparison to the globulin may cause the observed changes in the current study.

Present results revealed that the number of abomasal ulcers had a positive correlation with the α-globulin percent and a negative correlation with the serum γ-globulin concentration. The α-globulin band consists mainly of α 1-antitrypsin, α -2-macroglobulin, haptoglobulin, amyloid A and ceruloplasmin (Gruys *et al.*, 1994) which are considered as positive acute phase reactants and increase in response to inflammatory and traumatic conditions (Murata *et al.*, 2004). It is believed that the varying increase of the α-globulin reflects the intensity of the inflammatory response (Saleh *et al.*, 2008). The positive correlation between the number of abomasal ulcers and the α-globulin percent in the current study may be due to the moderate inflammatory reactions at the ulcer locations which were confirmed in the histopathological examination.

Although, Khadjeh and Razi-Jalali (2003) revealed that female water buffaloes have higher serum γ-globulin in comparison to male buffaloes and buffaloes up to 18 months of age had higher α-globulin and lesser β-globulin and γ-globulin in comparison to buffaloes older than 18 months, separate evaluation of both sexes and different age groups of buffaloes in the current study showed no major difference in the results of the comparison between affected and non-affected buffaloes.

It has been shown that measurement of the serum proteins concentrations can often be helpful in narrowing the list of diseases to be considered and in some cases in confirmation of disease diagnosis in different species of domestic animals (Nazifi *et al.*, 2009).

CONCLUSION

The cause of the findings of the current study and some contradictory findings regarding the changes of the serum protein electrophoresis in type I abomasal ulcers in water buffalo is not clear

and may be due to the effect of some factors such as age, sex, breed, pregnancy, geographic and dietary factors on the serum proteins profile in domestic animals. Present findings suggest that the serum protein electrophoresis may be useful in diagnosis of type I abomasal ulcers in water buffalo. However, it seems that more work is required on a larger number of animals before these findings can be used in practice.

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