

## THE SERUM PROTEIN ELECTROPHORETIC PATTERN AND ACUTE PHASE PROTEINS CONCENTRATIONS IN CALVES WITH CHRONIC RESPIRATORY DISEASES

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*The aim of this study was to evaluate the serum proteins electrophoretic pattern and the concentrations of acute phase proteins in calves with chronic bronchopneumonia. Twenty-eight calves with clinical symptoms of disease were included into the evaluation. Blood serum was analyzed for total protein concentrations (TP), total and relative values of serum protein fractions, albumin/globulin ratio (A/G) and the concentrations of selected acute phase proteins – haptoglobin (Hp), serum amyloid A (SAA), and fibrinogen (Fbg). The results recorded in sick animals were compared with those in 36 clinically healthy calves as control group. In calves suffering from chronic bronchopneumonia we found significantly higher total serum protein concentrations than in healthy calves ( $p < 0.001$ ). In sick calves a marked shift in the concentrations of most of the protein fractions was observed, with significantly higher mean total values of  $\alpha_1$ - ( $p < 0.001$ ),  $\beta_1$ - ( $p < 0.01$ ),  $\beta_2$ - ( $p < 0.001$ ), and  $\gamma$ -globulins ( $p < 0.001$ ) and relative mean values of  $\alpha_1$ - ( $p < 0.01$ ),  $\beta_2$ - ( $p < 0.01$ ), and  $\gamma$ -globulins ( $p < 0.001$ ). On the other hand, the concentrations of albumin and A/G ratios were in calves with respiratory diseases significantly lower than those measured in healthy ones ( $p < 0.001$ ). The mean concentrations of  $\alpha_2$ -globulins did not differ significantly between the two groups of animals. In the affected calves significantly higher mean values of Hp, SAA, as well as Fbg ( $p < 0.001$ ) were described. The presented results suggest a marked effect of chronic bronchopneumonia in calves on most of the evaluated protein profiles and acute phase protein variables with a marked shift in the albumin and globulin concentrations.*

*Key words: calves, electrophoresis, protein fractions, respiratory diseases*

## INTRODUCTION

Respiratory diseases and diarrhea are the leading causes of morbidity and mortality in calves, and may account for serious economic losses (Snowder *et al.*, 2006). Negative economic impact of respiratory diseases is associated with poor growth, reduction in live weight gain, reduced productive life span, treatment costs, and even death (McGuirk, 2008). In the case of dairy calf pneumonia, diagnosis and treatment are mainly based on the observation of clinical symptoms, such as depression and body temperature combined with specific disease signs. However, in many cases, the infected calves show only mild clinical symptoms that could be easily missed in a group of calves on a farm (Gänheim *et al.*, 2003). Therefore, there is a need for objective parameters that are suitable as indicators of health or disease in calf herds applicable in the laboratory diagnosis of diseases. Several blood parameters (e.g. total leukocyte count) have been introduced to indicate inflammatory diseases including respiratory diseases (Fulton *et al.*, 2002). However, the use of total leukocyte count to detect infection is not informative enough in cattle as is in many other species (Taylor, 2000). Objective parameters of animal health like the measurement of acute phase proteins and serum protein electrophoresis could be useful for identifying diseased animals.

Acute phase proteins are a group of proteins primarily synthesized by hepatocytes which concentrations change after infection, inflammation, tissue injury, trauma, surgery or immunological disorders, i.e. factors that affect the body's homeostasis (Murata *et al.*, 2004). In cattle, haptoglobin and serum amyloid A are diagnostically the most important acute phase proteins. These proteins have been found to increase in the serum of cattle with many different diseases, including respiratory tract diseases (Gänheim *et al.*, 2003). On the other hand, although it provides useful information on the pathological conditions associated with disorders of the protein profile, serum protein electrophoresis in cattle is a rarely used laboratory method. The serum protein electrophoresis is a laboratory technique used to separate serum proteins by size and electrical charge allowing thus the identification and quantification of protein fractions (Cerón *et al.*, 2010). Indications for serum protein electrophoresis in humans include hyperproteinaemia and suspicion of plasma cell neoplasia as well as investigation of hepatic and gastrointestinal diseases (Vavricka *et al.*, 2009). In veterinary medicine, previous reports described the serum protein electrophoretic pattern in small animals, goats, and horses, predominantly in hepatic, endocrine, infectious diseases as well as conditions resulting in monoclonal gammopathies (Janků *et al.*, 2011; Tappin *et al.*, 2011). However, the serum protein electrophoresis and identification of serum protein fractions in cattle with various organ diseases is less well documented, predominantly in chronic cases.

Therefore, the objective of this study was to determine whether chronic bronchopneumonia in calves causes changes in selected serum protein profile

variables and serum protein electrophoretic pattern. In addition, we evaluated the concentrations of selected acute phase proteins and their possible usefulness in the assessment of bovine respiratory diseases.

## MATERIAL AND METHODS

### *Animals and clinical examination*

Twenty-eight calves with clinical signs of chronic bronchopneumonia of various degrees were included into this study. The calves were of a Slovak spotted breed, low-land black spotted breed, or their crossbreeds. The age of the calves ranged from 4 to 6 months, and their body weight was 85 – 140 kg. The evaluated animals were submitted to the Clinic for Ruminants of the University of Veterinary Medicine and Pharmacy in Kosice by private veterinarians from three different conventional dairy farms. The same feeding and management regimes were applied to calves from these herds. At the clinic, the calves were housed individually, fed twice a day with free access to water.

After arrival to the clinic, all calves were thoroughly examined using standard clinical examination procedures oriented to the examination of the general health state (body temperature, feed intake, behaviour), and then specially to the respiratory system (Jackson and Cockcroft, 2002). The respiratory system was examined by visual inspection (breathing rate, nasal discharges, type of breathing, dyspnoea, dry or wet spontaneous cough) and auscultation (increased or decreased loudness of breathing sounds, bronchial sounds, abnormal breathing sounds – most commonly crackles, in some animals also wheezes and signs of laboured breathing with the mouth open). Into the evaluation we included calves with clinical signs of the disease manifested for more than 2 weeks despite the antimicrobial, antiinflammatory, and supportive therapy done by private veterinarians on the farm. The chronicity of the disease was defined from the patient history. The evaluated animals did not show any pathological lesions on other organ systems. To compare the evaluated variables between sick and healthy animals, 36 clinically healthy calves of the same age and breed in good general health without any obvious diseases were used as the control group.

### *Blood sample collection*

Blood samples for analyses of the evaluated parameters were taken once after initial clinical examination, when the clinical symptoms of the disease in sick animals were obvious. Blood samples were collected by a direct puncture of *v. jugularis* into serum gel separator tubes without anticoagulant. The separated serum was stored at -20°C until analyzed for total serum protein concentrations, identification of serum protein fractions, and for the determination of selected acute phase protein concentrations – haptoglobin (Hp, mg/mL) and serum amyloid A

(SAA,  $\mu\text{g/ml}$ ). For the determination of fibrinogen concentrations (Fbg,  $\text{g/L}$ ), blood samples were taken into special tubes with sodium citrate. Plasma was used for immediate analysis of Fbg after separation without storage.

#### *Laboratory analyses*

Total protein (TP,  $\text{g/L}$ ) concentrations in blood serum were assessed on automated biochemical analyser Alizé (Lisabio, France) by the biuret method using commercial diagnostic kits (Randox). Serum protein fractions were separated by zone electrophoresis on buffered agarose gel at pH 8.8 on an automated electrophoresis system Hydrasys (Sebia Corporate, France) using commercial diagnostic kits Hydragel 7 Proteine (Sebia Corporate, France) according to the procedure described by the manufacturer. Ten microliters of each serum sample were applied to the numbered sample wells on the agarose gel. The control serum (Control Serum Human Normal, Sebia Corporate, France) was included into each run of samples. The electrophoretic migration was performed for 15 minutes at  $20^\circ\text{C}$  constantly at 10 W, 40 mA, and 240 V. After migration, the gels were stained in amidoblack staining solution, and then destained by acidic solutions and dried completely. The electrophoretic gels were scanned, and the serum protein fractions were visualized and displayed on the densitometry system Epson Perfection V700 (Epson America Inc., California, USA) by light transmission and automatic conversion into an optical density curve presentation. Protein fractions were identified and quantified by computer software Phoresis version 5.50 (Sebia Corporate, France), and if necessary, corrected by visual inspection of the electrophoretogram.

Serum proteins were separated into the following fractions in the order from fastest to slowest mobilities: albumin,  $\alpha_1$  ( $\alpha_1$ )-,  $\alpha_2$  ( $\alpha_2$ )-,  $\beta_1$  ( $\beta_1$ )-,  $\beta_2$  ( $\beta_2$ )-, and gamma ( $\gamma$ )-globulins. The relative concentrations (%) of the protein fractions were determined as the percentage of the optical absorbance. Albumin: globulin ratios (A/G) were computed from the electrophoretic scan.

The concentration of haptoglobin was assessed using commercial colorimetric kits (Tridelta Development, Ireland) in microplates, based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH. Serum amyloid A was analyzed by the method of sandwich enzyme linked immunosorbent assay using commercial ELISA kits (Tridelta Development, Ireland) according to the procedure described by the manufacturer. The optical densities were read on the automatic microplate reader Opsys MR (Dynex Technologies, USA) at 630 nm for Hp, and 450 nm using 630 nm as reference for SAA. The determination of fibrinogen was performed using the semi-automatic 4-channel coagulometer Behnk CL-4 (Behnk Elektronik GmbH & Co., Germany) using commercial diagnostic kits (Diagon Kft, Hungary), based on the principle of electromagnetic detection of fibrin formation.

*Statistical analyses*

Arithmetic means ( $\bar{x}$ ) and standard deviations (SD) for each evaluated variable and group of calves were calculated using the descriptive statistical procedures. For acute phase proteins, median concentrations were calculated, too. Student's non paired t-test was used to compare the significance of differences in means between healthy and sick calves. All statistical analyses were performed using the computer programme GraphPad Prism V5.02 (GraphPad Software Inc., California, USA).

## RESULTS

The data referring to the total protein concentrations, the total and relative values of serum protein fractions, A/G ratios and the concentrations of evaluated acute phase proteins in healthy calves and calves affected by chronic bronchopneumonia, including the significance of differences in the means measured between the groups of animals, are given in Tables 1 and 2. The analysis of the individual concentrations of acute phase proteins in the two groups of animals, using the box and whisker plots is presented in Figures 1-3. The electrophoretic patterns of serum proteins in healthy calf and calf suffering from bronchopneumonia are shown in Figure 4.

Table 1. Concentrations of total serum proteins, serum protein fractions, and A/G ratios in healthy calves and calves suffering from bronchopneumonia (mean  $\pm$  SD).

Variable		Group of calves		P value
		Healthy	Sick	
Total proteins	g/L	69.6 $\pm$ 4.7	79.7 $\pm$ 12.0	< 0.001
Albumin	g/L	32.3 $\pm$ 4.1	27.4 $\pm$ 5.4	< 0.001
	%	46.4 $\pm$ 5.6	35.3 $\pm$ 9.1	< 0.001
$\alpha_1$ -globulins	g/L	9.7 $\pm$ 1.7	12.5 $\pm$ 2.3	< 0.001
	%	13.9 $\pm$ 2.2	15.9 $\pm$ 3.3	< 0.010
$\alpha_2$ -globulins	g/L	3.4 $\pm$ 0.9	3.4 $\pm$ 0.9	0.86
	%	4.9 $\pm$ 1.4	4.3 $\pm$ 1.3	0.078
$\beta_1$ -globulins	g/L	6.5 $\pm$ 1.0	7.9 $\pm$ 2.3	< 0.010
	%	9.4 $\pm$ 1.7	9.9 $\pm$ 2.2	0.317
$\beta_2$ -globulins	g/L	5.6 $\pm$ 1.4	8.1 $\pm$ 3.4	< 0.001
	%	8.0 $\pm$ 1.9	10.0 $\pm$ 3.2	< 0.010
$\gamma$ -globulins	g/L	12.3 $\pm$ 3.3	20.5 $\pm$ 9.0	< 0.001
	%	17.5 $\pm$ 4.0	24.8 $\pm$ 8.2	< 0.001
A/G ratio		0.88 $\pm$ 0.19	0.58 $\pm$ 0.23	< 0.001

Table 2. Concentrations of acute phase proteins in healthy calves and calves suffering from bronchopneumonia (mean  $\pm$  SD).

Variable	Group of calves		P value
	Healthy	Sick	
Haptoglobin (mg/mL)	0.14 $\pm$ 0.15	0.64 $\pm$ 0.69	< 0.001
Serum amyloid A ( $\mu$ g/mL)	23.1 $\pm$ 25.4	107.7 $\pm$ 48.7	< 0.001
Fibrinogen (g/L)	2.31 $\pm$ 0.46	3.44 $\pm$ 1.31	< 0.001

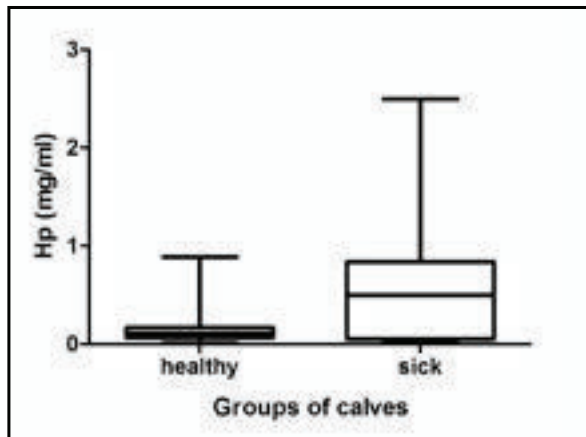


Figure 1. Distribution of Hp concentrations in healthy calves and calves suffering from chronic bronchopneumonia. The plots show the median (line within box), 25th and 75th percentiles (box), minimal and maximal values (whiskers)

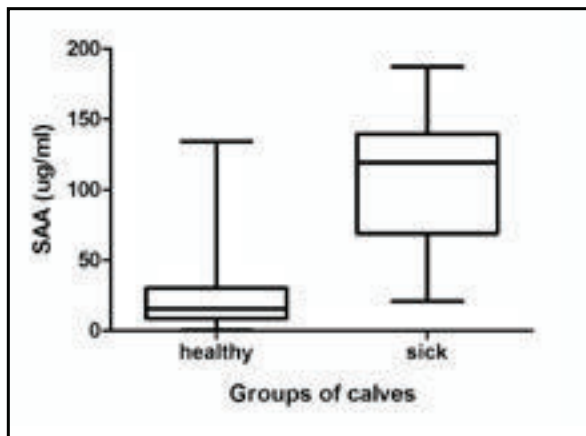


Figure 2. Distribution of SAA concentrations in healthy calves and calves suffering from chronic bronchopneumonia. The plots show the median (line within box), 25th and 75th percentiles (box), minimal and maximal values (whiskers)

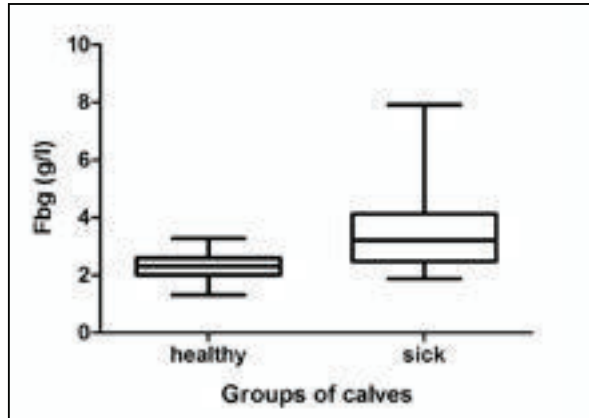


Figure 3. Distribution of Fbg concentrations in healthy calves and calves suffering from chronic bronchopneumonia. The plots show the median (line within box), 25th and 75th percentiles (box), minimal and maximal values (whiskers)

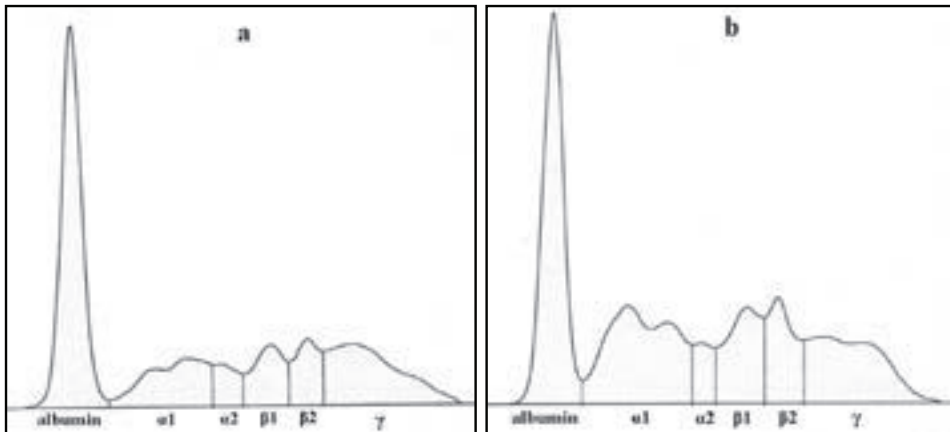


Figure 4. Electrophoretic pattern of serum proteins in a healthy calf (a) and a calf suffering from chronic bronchopneumonia (b)

The mean total serum protein concentrations in calves suffering from chronic bronchopneumonia were significantly higher than in healthy animals ( $p < 0.001$ , Table 1). Significant differences between the evaluated groups of calves were found also for the total and relative concentrations of albumin, with values in sick calves being significantly lower than in healthy animals ( $p < 0.001$ ). An opposite trend was observed in the total and relative concentrations of  $\alpha_1$ -globulins with significantly higher mean values in calves with respiratory diseases ( $p < 0.001$  and  $p < 0.01$ , respectively). The mean concentrations of  $\alpha_2$ -globulins did not differ significantly between the two groups of animals. Significant effects of chronic

respiratory diseases were recorded also in  $\beta_1$ - and  $\beta_2$ -globulin fractions with higher mean total concentrations in sick calves ( $p < 0.01$  and  $p < 0.001$ , respectively) and higher relative  $\beta_2$ -globulin fractions ( $p < 0.01$ ). Similarly, the total and relative concentrations of  $\gamma$ -globulins differed significantly between the evaluated groups of calves with higher values in animals with bronchopneumonia ( $p < 0.001$ ). The mean A/G ratio in calves with chronic respiratory diseases was significantly lower ( $p < 0.001$ ).

The average Hp concentration in calves suffering from chronic respiratory diseases was significantly higher compared with healthy animals ( $p < 0.001$ , Table 2). While the median Hp concentration in healthy calves was 0.11 mg/mL and the individual values ranged from 0.01 to 0.89 mg/mL, in calves with clinical symptoms of respiratory diseases we recorded higher median Hp concentration (0.50 mg/mL) as well as markedly wider range of individual values with maximal Hp concentration of 2.50 mg/mL (Fig. 1). Significant differences between healthy and sick calves were found in the mean concentrations of SAA, with markedly higher concentrations in calves affected by chronic respiratory diseases ( $p < 0.001$ ). The median concentration of SAA in healthy calves was 15.45  $\mu\text{g/mL}$  and the individual values ranged from 0.08 to 134.00  $\mu\text{g/mL}$  (Fig. 2). The median SAA concentration in calves with respiratory signs was noticeably higher (119.00  $\mu\text{g/mL}$ ), and the range of individual values was wider (from 20.80 to 187.00  $\mu\text{g/mL}$ ). A more detailed analysis of SAA concentrations showed that 50% of the individual values in healthy calves were within the range of 8.60 to 30.15  $\mu\text{g/mL}$  and in sick calves of 68.95 to 139.50  $\mu\text{g/mL}$ . Plasma mean concentrations of fibrinogen were significantly higher in sick calves ( $p < 0.001$ , Table 2). The median concentrations of Fbg in healthy and sick animals were 2.30 g/L and 3.21 g/L, respectively, and the individual values ranged from 1.32 to 3.27 g/L and 1.87 – 7.90 g/L, respectively (Fig. 3).

Changes reflecting the effect of chronic bronchopneumonia on the serum protein electrophoretic pattern are presented in Figure 4.

## DISCUSSION

In humans, serum protein electrophoresis is a common technique of laboratory diagnosis used to identify patients with multiple myeloma and to investigate serum dysproteinaemias, gastrointestinal, as well as liver diseases (O'Connell *et al.*, 2005, Vavricka *et al.*, 2009). In small animal and equine medicine, indications for performing the serum protein electrophoresis have largely focused on the investigation of hyperproteinaemia, hepatic diseases, and screening for gammopathies and immunodeficiencies (Mair *et al.*, 1993; Tappin *et al.*, 2011). On the other hand, there are only scarce data regarding the serum protein electrophoretic pattern in cattle with various organ diseases, and the studies were performed some years ago. Some data were presented earlier in cows suffering from traumatic gastritis, gastro-phrenitis, and traumatic peritonitis, with a decrease of the albumin and increase of globulin concentrations (Samadieh *et al.*, 1978;



Yoshida, 1986). However, sparse information regarding serum electrophoretic pattern and changes in albumin and globulin fractions in cattle with respiratory diseases has been published.

In the presented study the results showed a significant effect of respiratory diseases in calves on the concentrations of most of serum protein fractions. The relevance of protein fractions for early selection of treatment and detection of calves with bronchopneumonia was investigated earlier by Humblet *et al.* (2004). The aforementioned study suggested significant differences between diseased and recovered calves for total serum proteins, albumin,  $\beta_1$ - and  $\gamma$ -globulins. All these serum proteins increased from the diseased to the recovered status, except  $\gamma$ -globulins, which decreased. On the other hand, in the study presented by Humblet *et al.* (2004), the concentrations of  $\alpha_1$ - and  $\alpha_2$ -globulins in healthy calves and calves with bronchopneumonia were approximately similar. In our study, the comparison of serum protein fractions between healthy and sick animals showed significantly higher concentrations of  $\alpha_1$ -globulins in calves with bronchopneumonia. According to Carapeto *et al.* (2006), acute inflammatory diseases usually lead to an increase in some of the proteins in the  $\alpha_1$ -globulin fraction. Jawor *et al.* (2008) reported that serious inflammatory conditions are associated with higher concentrations of  $\alpha$ -globulins, and that this increase is caused by the fact that the majority of acute phase proteins (haptoglobin, ceruloplasmin,  $\alpha_1$ -acid glycoprotein,  $\alpha_1$ -antitrypsin) occurs in this fraction. Our results suggest that not only acute inflammatory diseases, but also chronic infections may be associated with changes in the  $\alpha$ -globulin fraction, as we found in calves with chronic bronchopneumonia significantly higher concentrations of  $\alpha_1$ -globulins compared to healthy animals. Tymchak (2010) reported also that chronic infections may produce an increase in globulin fractions, predominately in  $\gamma$ -globulins, but also in the  $\alpha$ - and  $\beta$ -globulin fractions, which is accompanied by a decrease of albumin. On the other hand, Carapeto *et al.* (2006) evaluated horses suffering from different diseases with a chronic inflammatory component, and they found similar average values for  $\alpha$ -globulin subfractions in healthy, as well as sick horses, although the  $\alpha_1$ -globulin concentration was slightly higher in sick animals. Therefore, further studies would be helpful to clarify the effect of chronic respiratory diseases on the concentrations of globulin fractions and their changes during the disease process, as these findings are clinically important for identifying the inflammatory process as active, which may be helpful in achieving a final diagnosis and specifying appropriate treatment.

The  $\alpha$ -globulin fraction includes many of the acute phase proteins (ceruloplasmin, haptoglobin,  $\alpha_1$ -acid glycoprotein, some lipoproteins), which have great potential as biomarkers of many economically important diseases. The presented results suggest that chronic respiratory-tract diseases of calves induce acute phase response as measured by higher concentrations of haptoglobin, as well as serum amyloid A compared with clinically healthy animals. These results are in agreement with previous findings which reported that the concentrations of

these proteins rise in cattle with respiratory diseases (Wittum *et al.*, 1996; Gänheim *et al.*, 2007). According to Horadagoda *et al.* (1999) chronic inflammation can be identified as a consecutive series of separate inflammatory stimuli with higher serum concentrations of acute phase proteins, which could explain the increase of  $\alpha$ -globulin. Therefore, the increase in  $\alpha$ -globulins in calves affected by chronic respiratory diseases may be a consequence of the increase of acute phase proteins from this fraction. Carapeto *et al.* (2006) evaluated the proteinograms in horses suffering from inflammatory diseases, and they stated that for the interpretation of the obtained results must be considered that these diseases cause an inflammatory response sufficient for the detection of the increase of acute phase proteins by zone electrophoresis. However, seeing that the globulin fractions include a number of individual proteins, additional studies are needed to identify the individual proteins associated with higher relative concentrations of  $\alpha_1$ -globulins in calves suffering from respiratory diseases. High resolution electrophoresis is designed for better resolution and multifractionation of serum proteins allowing researchers to separate single proteins (Abate *et al.*, 2000). This technique, as well as quantification of acute phase proteins, could be helpful in determining the main proteins responsible for higher relative concentrations of  $\alpha_1$ -globulins in calves with signs of respiratory diseases.

The presented study indicated in calves with chronic respiratory-tract diseases significantly higher concentrations also for  $\beta_1$ - and  $\beta_2$ - globulins. An opposite trend was observed by Humblet *et al.* (2004) with higher concentrations of  $\beta_1$ - globulins in healthy calves compared with calves suffering from bronchopneumonia. Similar findings were reported by Carapeto *et al.* (2006) in horses affected by diseases with a chronic inflammatory component. According to O'Connell *et al.* (2005), the increase in the concentration of  $\beta$ -globulins occurs with inflammation, neoplasia, and various metabolic disorders, including chronic infections that may also produce the increase found in  $\beta$  fractions. However, the increase in  $\beta$ -globulins alone is infrequent in most species and are accompanied by the increases in  $\gamma$ -globulins (Eckersall, 2008). On the other hand, there is a concomitant decrease in albumin as a result of its decreased synthesis. These data are in agreement with our findings, as in calves suffering from chronic respiratory diseases we found significantly higher concentrations of  $\gamma$ -globulins, but significantly lower values of albumin. Seeing that the gamma region is composed predominantly of antibodies of the IgG type, higher concentrations of  $\gamma$ -globulins obtained in calves with chronic respiratory diseases may reflect the response of the organism to inflammation. Taylor *et al.* (2010) indicated that the increases in globulin fractions, particularly of the  $\gamma$ -globulins are associated with chronic antigenic stimulation. Stockholm and Scott (2008) stated that decreased albumin and an increase in globulin concentrations is the most common pattern in animals subjected to inflammatory diseases, which reflect the compensatory reduction in albumin concentrations to maintain oncotic pressure and viscosity. Moreover, since albumin is a negative acute phase protein, the aforementioned factors probably

contributed to significantly lower concentrations of albumin in calves suffering from chronic respiratory diseases observed in the present study. This shift in the albumin and globulin concentrations resulted in significantly lower A/G ratio in calves affected by chronic respiratory diseases suggesting the significance of A/G ratio in the classification of the electrophoretic profile and identification of dysproteinemias. However, according to Alberghina *et al.* (2010), the A/G ratio must be interpreted cautiously with attention to the changed part of the ratio.

Regarding the concentrations of fibrinogen, the usefulness of its plasma concentrations in cattle has been demonstrated mostly by the diagnosis of traumatic conditions, monitoring of postoperative complications, e.g. peritonitis, as well as by the differentiation of traumatic reticuloperitonitis from other gastrointestinal disorders (Jafarzadeh *et al.*, 2004). The results presented show that the determination of the concentrations of Fbg may be useful also in the monitoring of respiratory diseases, because in calves suffering from chronic respiratory diseases we found significantly higher concentrations of Fbg than in clinically healthy animals. Similar findings were obtained earlier by Humblet *et al.* (2004) in growing calves, which showed that early determination of haptoglobin together with fibrinogen allows the identification of about 70% of the calves suffering from bronchopneumonia that required antibiotic and anti-inflammatory drugs. Although most of the acute phase proteins are  $\alpha$ -globulins, fibrinogen migrates in the  $\beta$  region or between the  $\beta$  and  $\gamma$  regions, and plasma protein electrophoresis is needed to demonstrate Fbg (Meyer and Harvey, 2004). Fibrinogen in the serum specimens can be misinterpreted in protein electrophoresis as monoclonal immunoglobulins (Qiu *et al.*, 2003). In our study, the concentrations of Fbg in plasma samples were determined using the automatic coagulometer based on the principle of electromagnetic detection of fibrin formation. The exact demonstration and visualisation of individual proteins present in each area of the electrophoretic trace in the study was not performed. Therefore, determination of the individual protein changes represents a possible area for future investigations.

In conclusion, the presented study suggests that chronic respiratory diseases in calves may affect the serum protein electrophoretic pattern with a marked shift in the albumin and globulin concentrations. The most significant differences between calves suffering from chronic respiratory-tract diseases and healthy animals were observed in the concentrations of total proteins, albumin,  $\alpha_1$ -,  $\beta_2$ -, and  $\gamma$ -globulin fractions. While the concentrations of TP,  $\alpha_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins were significantly higher in calves with respiratory diseases, the albumin values and A/G ratios were significantly lower in the affected calves. Seeing that the changes in the serum protein electrophoretic pattern in calves with chronic respiratory diseases are not sufficiently evaluated, the obtained data could be useful for clinicians for the determination of the health status and the evaluation of pathological changes in the affected animals and better evaluation of the systemic status providing a basis for further specific laboratory investigations. Moreover, the presented study showed in calves suffering from chronic bronchopneumonia markedly higher concentrations

of the evaluated acute phase proteins – haptoglobin, serum amyloid A as well as fibrinogen, which probably influenced also the changes observed in the serum protein electrophoretic pattern in the affected animals. Thus, these results support the usefulness of acute phase proteins measurements in the monitoring of animals with respiratory diseases. However, to determine the individual proteins responsible for the changes in the serum protein electrophoretic pattern in calves suffering from chronic respiratory diseases further investigations using high resolution electrophoresis are needed to yield satisfactory results.

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## ELEKTROFORETSKE KARAKTERISTIKE PROTEINA KRVNOG SERUMA I KONCENTRACIJA PROTEINA AKUTNE FAZE KOD TELADI SA HRONIČNIM RESPIRATORNIM OBOLJENJIMA

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### SADRŽAJ

Cilj ovih ispitivanja je bio da se odrede elektroforetske karakteristike serumskih proteina i koncentracija proteina akutne faze kod teladi sa hroničnom bronhopneumonijom. U ispitivanje je bilo uključeno 28 teladi sa kliničkim znacima bolesti. U krvnom serumu je određivana koncentracija ukupnih proteina (TP), apsolutne i relativne vrednosti proteinskih frakcija albuminsko/globulinski količnik (A/G) i koncentracije odabranih proteina akutne faze: haptoglobina (Hp), serumskog amiloida A (SAA) i fibrinogena (Fbg). Rezultati registrovani kod obolele teladi su zatim upoređivani sa vrednostima istih parametara 36 klinički zdravih životinja iz kontrolne grupe. Telad obolela od hronične bronhopneumonije imala je značajno višu koncentraciju ukupnih proteina u poređenju sa zdravim jedinkama ( $p < 0,001$ ). Osim toga, kod obolele teladi su bile veće apsolutne koncentracije  $\alpha_1$ - ( $p < 0,001$ ),  $\beta_1$ - ( $p < 0,01$ ),  $\beta_2$ - ( $p < 0,001$ ) i  $\gamma$ -globulina ( $p < 0,001$ ) kao i njihove relativne vrednosti ( $\alpha_1$ -  $p < 0,01$ ,  $\beta_2$ -  $p < 0,01$ , i  $\gamma$ -globulina  $p < 0,001$ ). Istovremeno je koncentracija albumina bila manja kod obolelih kao i A/G količnik u poređenju sa vrednostima kod zdravih ( $p < 0,001$ ). Srednja koncentracija  $\alpha_2$ -globulina nije se razlikovala između grupa. Kod obolele teladi je dokazana značajno viša koncentracija Hp, SAA kao i Fbg ( $p < 0,001$ ). Ovi rezultati ukazuju na značajan uticaj hronične bronhopneumonije teladi na većinu određivanih parametara proteinskog profila kao i na proteine akutne faze. Najveće promene su uočene u koncentraciji albumina i globulina.