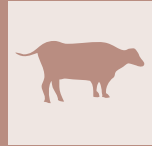


Electrophoretic profile of serum proteins of cattle with bronchopneumonia



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SUMMARY

Bovine Respiratory Diseases (BRD) is a common devastating disease for cattle breeding, both calves, beef and dairy cows, but chiefly in calves. Our study, enrolling 200 cattle, aimed to determine the serum protein fractions and Albumin to Globulin ratio in two groups of cattle (157 healthy cattle and 43 with clinically diagnosed bronchopneumonia) from different age categories, beef and dairy cows, diverse physiological status, both male and female, and to evaluate the feasibility of using these biochemistry parameters as diagnostic biomarkers, in order to reduce a various para-clinical examinations. All the 200 obtained blood samples were analyzed through serum protein electrophoresis on buffered agarose gel at pH 9.1 using an automated electrophoresis system Hydrasys (Sebia Corporate, and we determined one of selected acute phase protein concentrations: haptoglobin. results were expressed as mean $\pm$ standard deviation (SD), The Shapiro-Wilk test was used to check normality before performing parametric tests such as Student test and Analysis of variance. One-way nonparametric analysis of variance was applied to compare groups (Kruskal-Wallis test), when the assumptions of one-way ANOVA were not met. Regardless to age, sex and physiological status, serum protein concentrations were found to be significantly higher ($p < 0.001$) in cattle suffering from bronchopneumonia, as compared to healthy ones. the concentrations of γ -globulins differed significantly between the evaluated groups of bovines with higher values in animals with bronchopneumonia ($p < 0.01$), also for β globulins. Haptoglobin concentrations greater than 0.41g /l were observed in ten (10) cows from the same breeding (5%) diagnosed with bronchopneumonia. In unhealthy cattle a marked shift in the concentrations of most protein fractions was observed, with significantly higher mean values of α_1 -globulins ($p < 0.001$). Given that the α -globulin fraction includes many of the acute phase proteins such as haptoglobin. Also, serum proteins electrophoresis measurements in the monitoring of animals with respiratory diseases. Therefore, we could evaluate EPS instead of acute phase protein to determine the individual proteins responsible for changes in the electrophoretic pattern of serum proteins in cattle with bronchopneumonia.

KEY WORDS

Bronchopneumonia, cattle, Serum Proteins Electrophoresis, Haptoglobin.

INTRODUCTION

Respiratory disorders are considered as the second most important cause of death in calves and a main cause of economic loss in the cattle farming worldwide, they are caused by various ubiquitous pathogens in cattle populations, including viruses (Bovine Herpes Virus-1, Bovine Viral Diarrhea, Bovine Respiratory Syncytial Virus) or/and bacteria (*Mannheimia haemolytica*, *Pasteurella multocida*, *Haemophilus somnus*, *Mycoplasma spp.*) (1). These infectious agents most often occur following environmental stress represented by various factors such as transport, weaning, promiscuity, poor ventilation, crowding, bad weather condition, dust, poor ventilation, malnutrition, etc., which impact negatively the bovine immune sys-

tem (2,3) and causes significant economic losses (4). Isolation of the responsible causal agents, biochemical, hematological and immunological analyzes had been widely applied in epidemiological investigations, both in fundamental and applied research to study the pathogenesis of the disease. However, most of these methods, available for current veterinary practice, are rarely used for the diagnosis of bovine bronchopneumonia (5). Often, the clinician diagnoses broncho-pneumonia on the basis of the observation of clinical signs, including depression, hyperthermia, cough, dyspnea, etc., however these symptoms most often evolve insidiously (6). Consequently, there is a need for reliable biochemistry biomarkers suitable for early diagnostic of bronchopneumonia (7). Various blood parameters, such as haptoglobin and analytic analysis like: Serum Protein electrophoresis (SPE), have been introduced to evaluate health conditions chiefly low-grade inflammatory diseases including respiratory disorders (8, 9). Indeed, these two methods could be useful for identifying diseased animals. The main advantages of SPE its low

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cost and easy handling, being less time consuming. Furthermore, it is well established that all most acute phase proteins (APP) like haptoglobin migrate on α_1 globulins zone (10, 11). It has been established, by previous investigations, that the distribution and concentration, both relative and absolute, of blood proteins are affected by many diseases. The identification and quantification of serum protein fractions makes it possible to identify animals carrying an alteration in the serum protein profile, which may reflect responses to changes in homeostasis or disease (12). For this reason, our biochemistry study on serum protein fractions aimed to determine how bovine bronchopneumonia affects blood protein profile, and why it can substitute the use of the haptoglobin in diagnosis of inflammatory respiratory affections. The importance of this study lies in the reduction of various para-clinical examinations known in respiratory bovine medicine and searching for a quick and inexpensive tool.

MATERIAL AND METHODS

Ethical approval: The research was approved by the Research Ethics Committee of our scientist university committee (Ref: 19/CS 04). Animal handlings were observed to ensure no discomfort or pain to animal during sampling.

Animals: Two hundred (200) cattle from the middle north of Algeria, distributed in 16 breeding sites, were enrolled in the study without predetermination and blood sampled. The fourth-three (43) naturally infected cattle (21,5%) with clinical bronchopneumonia disorder, ranging from 6 months to more than nine (9) years, of different breed, physiological status and sex, were used as animal material. 157 (78,5%) cattle without clinical pneumonia symptoms were determined as the healthy control group. Three factors, namely age category, physiological status and presence absence of Bronchopneumonia were considered in the study. The age category was divided in three groups: less than two years, 2 to 7 years and more than 7 years. The physiological status was categorized as follows: calves, heifers, dry cows, dairy cows, pregnant cows, bulls and fattening cattle.

Clinical examination : The group with bronchopneumonia were detected through standard clinical examination procedures: high body temperature, anorexia, behavior, with special focus on the respiratory system: by visual inspection (breathing rate, nasal discharges, type of breathing, dyspnea, dry or wet spontaneous cough, wheezes and signs of problematic breathing with the mouth open), auscultation (increased or decreased loudness of breathing sounds, bronchial sounds, abnormal breathing sounds most commonly crackles). Animals with normal body temperature, respiratory rate and normal lung auscultation, without cough nor breathing difficulty were categorized as healthy group (without broncho-pneumoniae or others suspicious symptoms). The animals affected by others pathology are rejected from this study.

Sample and sampling: Blood samples were collected by a direct puncture of *v. jugularis* into serum gel separator tubes without anticoagulant and other with heparin for haptoglobin measurement (Vacutainer® type). All will be placed in a cooler. Immediately, the blood sample is centrifuged at 3000 rpm for 10

minutes. After that, the serum taken is frozen (-4°C).

Laboratory exams: Total protein (TP, gr/l) and haptoglobin (Hp, mg/ml), one of the main acute phase proteins concentrations in blood plasma were assessed on automated biochemical analyzer (COBAS 6000. ROCHE^{INC}, designed by Hitachi) by the biuret method and immune-turbidimetry, respectively. Serum protein fractions were separated by zone electrophoresis on buffered agarose gel (14) at pH 9.1 using an automated electrophoresis system Hydrasys (Sebia Corporate, France) using commercial diagnostic kits Hydragel 5 Proteins (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°C constantly at 10 W, 40 mA, and 240 V. After migration, the gels were stained in Amidoschwartz 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 Hydrasys SEBIA, Gel Scan, DVSE SEBIA by light transmission and automatic conversion into an optical density curve presentation. Protein fractions were identified and quantified by computer software PHORESIS SEBIA version 5.50 (Sebia Corporate, France), and if necessary, corrected by visual inspection of the electrophoresis graph, this process allows five separate protein fractions to be obtained (Figure 3).

Statistical analyses: Both the main and the combined effects of the factors studied on the serum protein fractions were explored using multifactorial parametric analysis of variance. The Tukey multiple-comparison test was applied for post hoc comparison. Data were analysed using the R (15) statistical software (Version 4.3.1). All obtained results were expressed as mean $\pm$ standard deviation (SD). The Shapiro-Wilk test was used to check normality before performing parametric tests such as Student test and Analysis of variance. One-way nonparametric analysis of variance was applied to compare groups (Kruskal-Wallis test), when the assumptions of one-way ANOVA were not met. proteins fractions identified and measured by electrophoretic separation were served as quantitative response variables for testing hypothesis. The factor considered is especially the presence/absence of clinical broncho-pneumoniae (binary variable).

RESULTS

Results referring to concentrations of serum protein fractions in the two groups of sampled animals, expressed as means and standard deviations, including the level of significance of the differences in means between the groups, were summarized in Table 1. The mean total serum protein concentrations in cattle suffering from broncho-pneumonia were significantly higher than in healthy animals ($p < 0.01$, Table 1) in total agreement with other researches (14, 16). No Significant differences between the group with broncho-pneumonia and without broncho-pneumonia group were found regarding the concentrations of albumin, although its value in cattle with broncho-pneumonia was apparently lower than in healthy animals (with-

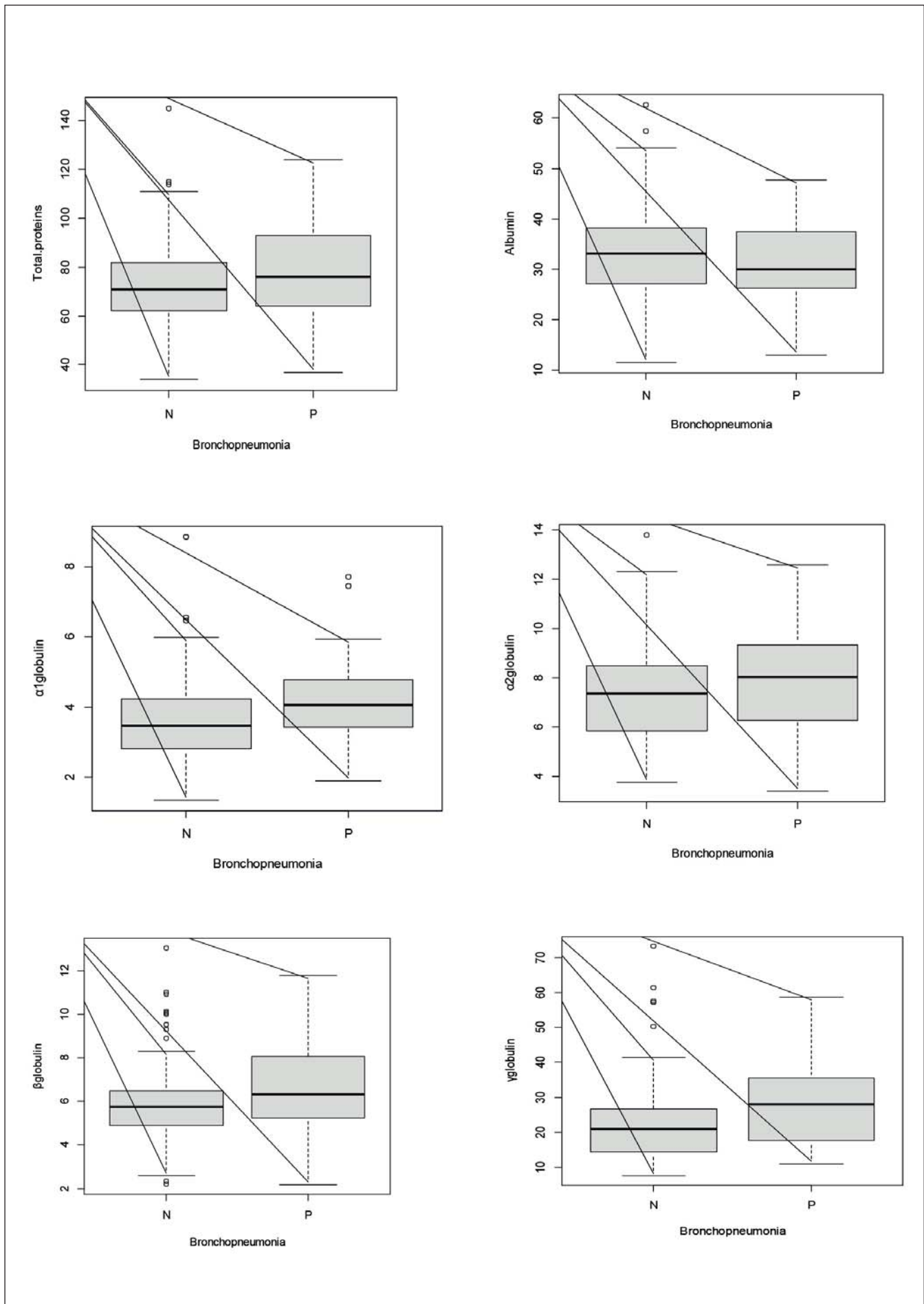


Figure 1 - Series of Boxplots exploring the evaluated serum protein fractions according to two groups of cattle, healthy and diseased cattle (TP: total proteins; Alb: Albumin; $\alpha_1,2$: alphaglobulins; β : bétaglobulins, γ : gamma globulins).

Table 1 - Concentrations of total serum proteins, serum protein fractions in healthy cattle and cattle suffering from bronchopneumonia (mean $\pm$ SD).

Proteins	(n ₁ =157)		(n ₂ =43)		Difference p-value
	Mean	SD	Mean	SD	
TP(gr/l)	72.04430	17.61056	79.07143	20.37142	0.01807
Alb(gr/l)	32.69089	8.43854	31.21119	8.71006	0.3951
α 1G(gr/l)	3.569494	1.098773	4.201429	1.178202	0.00157
α 2G(gr/l)	7.383354	2.004881	8.034762	2.27187	0.07262
β G(gr/l)	5.825063	1.647212	6.660000	1.976845	0.01002
γ G(gr/l)	22.57589	10.68037	28.96048	12.96698	0.002206
Alb/G	0.904073	0.2860236	0.710545	0.2487199	0.00003898

n₁: Healthy cattle (without others suspicious symptoms) ; n₂: Cattle suffering from BP; TP: total proteins; Alb: Albumin; α 1,2: alphaglobulins; β : bétaglobulins, γ : gamma globulins

out others suspicious clinical signs) ($p=0.395$), the same result is found in previous investigations (14, 17). An opposite trend was observed in the total and the concentrations of α_1 -globulins (Tables 1 and 4) and Albumin to globulin ratio (Tables 1 and 2) with significantly higher mean levels in cattle suffering from respiratory diseases ($p<0.001$), which agrees with previous researches (12, 14, 16). Furthermore, the multifactorial analysis of variance showed that, in addition to bronchopneumonia, only the age category had a significant effect ($p = 0.030$) on the concentration of serum total proteins (Table 3). The concentrations of α_2 -globulins did not differ significantly between the two groups of animals (tables 1 and 5). Significant effect of bronchopneumonia on β globulin fraction levels (Tables 1 and 6) was observed, with higher mean level in sick animals ($p<0.01$), like the result reported by (18, 19). Similarly, the concentrations of γ -globulins (Table 1) differed significantly between the evaluated groups of bovine with higher values in animals with bronchopneumonia ($p<0.01$) the same result was found with others authors (14, 17). Besides, the multifactorial analysis of variance showed that, in addition to bronchop-

neumonia, only the age category had a highly significant effect ($p=0.005$) on the concentration of β globulin fraction. The mean albumin to globulin ratio (Tables 1 and 2) in cattle clinically diagnosed with broncho-pneumonia was highly significant ($p < 0,01$, Table 2). Haptoglobin concentrations greater than physiological value was observed in ten (10) cows from the same breeding (5%) diagnosed with bronchopneumonia (between 40mg/l and 50mg/l) this feature has been reported in other scientific works (8, 20). These haptoglobin levels agree with that found by other researchers who recorded values ranging from: 0 to 60-70 mg / l (8, 9). However, we did not detect any increase in other cases of bronchopneumonia (10 out of 43 cases) despite of specifics symptoms, the recorded levels were the same as that of healthy cattle.

DISCUSSION

In this study, the results showed a significant effect of bovine respiratory diseases regardless to age or sex or physiological sta-

Table 2 - Table of multifactorial analysis of variance exploring all effects of 3 factors on albumin to globulin ratio).

Protein fraction	Factors	F value	p-value
Albumin to globulin ratio	Age category	1.9703	04243
	Bronchopneumonia	8.3963	0.004243 **
	Physiological status	2.0700	0.059059
	Age *Bronchopneumonia	1.6367	0.182649
	Age * Physiological status	0.9166	0.471568
	Bronchopneumonia*Physiological status	0.0152	0.999540
	All factors combined	0.5420	0.582553

Table 3 - Table of multifactorial analysis of variance exploring all effects of 3 factors on total proteins.

Protein fraction	Factors	F value	p-value
Total proteins	Age category	3.0478	0.03014 *
	Bronchopneumonia	1.9539	0.16394
	Physiological status	0.7337	0.62306
	Age *Bronchopneumonia	2.0091	0.11446
	Age * Physiological status	1.0066	0.41540
	Bronchopneumonia * Physiological status	0.9999	0.40913
	All factors combined	1.1901	0.30665

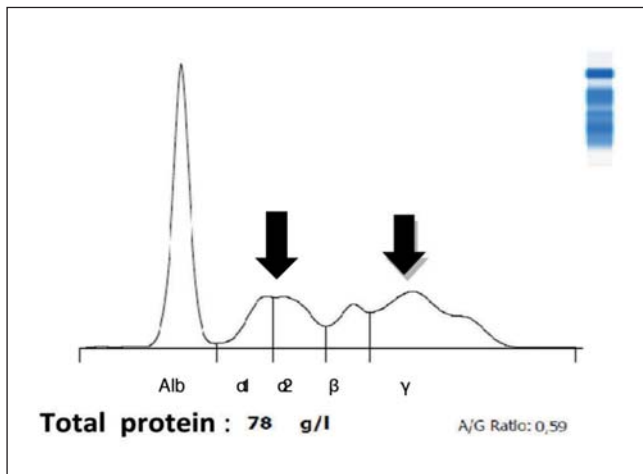


Figure 2 - A plot of Heifer, suffering from Broncho- pneumonia associated with liver disease revealed by an increase alpha 1, alpha2-globulins, also with Beta and Gamma globulins (TP: total proteins; Alb: Albumin; α 1,2: alphaglobulins; β : bétaglobulins, γ : gamma globulins).

tus, on the concentrations of the most serum protein fractions: there were significant differences between diseased and healthy cattle, mainly total serum proteins, α ₁, β - and γ -globulins (see Figure 1, and 2). All these serum proteins increased in diseased animals while α ₂-globulins and Albumin not changed. On the other hand, in the other study, the concentrations of these different fractions in healthy bovine and broncho-pneumonia were approximately similar (9, 16). However, in our study, the comparison of serum protein fractions between healthy and sick animals showed significantly higher concentrations of α ₁-globulins in bovine with bronchopneumonia. Acute inflammatory diseases usually lead to an increase in some serum proteins such as α ₁-globulin fraction (10, 21). Janciauskiene (23), reported that serious inflammatory conditions are associated with

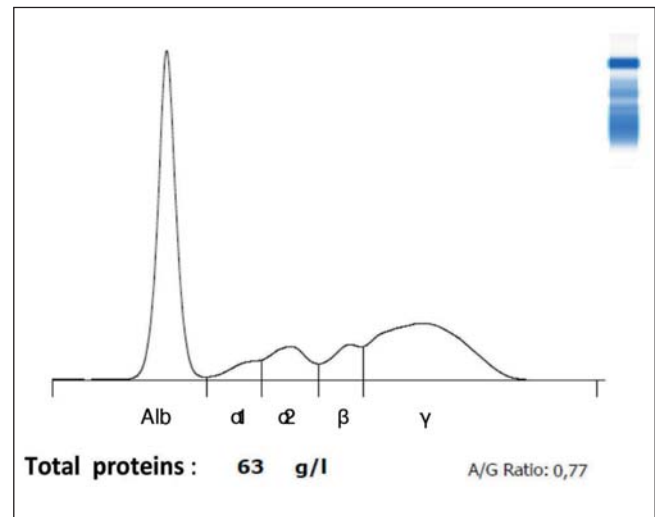


Figure 3 - A normal plot of calf, five years old (TP: total proteins; Alb: Albumin; α 1,2: alphaglobulins; β : bétaglobulins, γ : gamma globulins).

higher concentrations of α ₁-globulins, and that this increase is caused by the fact that the majority of acute phase proteins (haptoglobin, ceruloplasmin, α ₁-acid glycoprotein, α ₁-antitrypsin) are parts of this fraction. However, Tóthová (14), assume that not only acute inflammatory diseases, but also chronic infections may be associated with changes in the α -globulin fraction in calves. The present results showed that the bovine bronchopneumonia diseases could induce acute phase response, some higher concentrations of haptoglobin were recorded in 10 cases out of 43 diseased cattle (21.28%). These results were in partial agreement with previous findings which reported that the concentrations of these proteins rise in cattle with respiratory diseases (8, 20). Chronic inflammation can be identified as a result of inflammatory stimuli with an impact in serum

Table 4 - Table of multifactorial analysis of variance exploring all effects of 3 factors on α 1 Globulin.

Protein fraction	Factors	F value	p-value
α 1 Globulin fraction	Age category)	0.9891	0.399285
	Bronchopneumonia	7.8312	0.005715 **
	Physiological status	0.4662	0.832703
	Age *Bronchopneumonia	0.6775	0.566923
	Age * Physiological status	1.1126	0.355447
	Bronchopneumonia * Physiological status	0.3734	0.827395
	All factors combined	0.6934	0.501238

Table 5 - Table of multifactorial analysis of variance exploring all effects of 3 factors on α 2 Globulin.

Protein fraction	Factors	F value	p-value
α 2 Globulin fraction	Age category	1.1175	0.3294
	Bronchopneumonia	1.5721	0.2116
	Physiological status	0.8702	0.5623
	Age *Bronchopneumonia	0.4909	0.6129
	Age * Physiological status	1.8535	0.1207
	Bronchopneumonia * Physiological status	0.8350	0.5046
	All factors combined	0.4363	0.5098

Table 6 - Table of multifactorial analysis of variance exploring all effects of 3 factors on β Globulin.

Protein fraction	Factors	F value	p-value
β Globulin fraction	Age category	1.8414	0.1414
	Bronchopneumonia	6.2020	0.0137 *
	Physiological status	0.3615	0.9024
	Age *Bronchopneumonia	1.9268	0.1270
	Age * Physiological status	0.1952	1.4904
	Bronchopneumonia * Physiological status	1.0204	0.3983
	All factors combined	0.7655	0.4667

Table 7 - Table of multifactorial analysis of variance exploring all effects of 3 factors on γ Globulin.

Protein fraction	Factors	F value	p-value
γ Globulin fraction	Age category	4.3265	0.005707 **
	Bronchopneumonia	3.5705	0.060476
	Physiological status	0.8593	0.526095
	Age *Bronchopneumonia	1.0409	0.375873
	Age * Physiological status	0.5368	0.748192
	Bronchopneumonia * Physiological status	0.8468	0.497302
	All factors combined	0.0639	0.938144

concentrations of acute phase proteins, which could explain the increase of α_1 -globulin (16, 22). Therefore, the increase in α_1 -globulins, in cattle affected by bronchopneumonia may be a consequence of the increase of acute phase proteins comprised in this fraction. Additional studies are needed to identify the individual proteins associated with higher relative concentrations of α_1 -globulins in bovine suffering from respiratory diseases. The present study indicated in cattle affected by bronchopneumonia diseases significantly higher concentrations also for β globulins (see table 1). In other research, the authors explained that the increase in the concentration of β -globulins occurs as a result of inflammation, neoplasia and various metabolic disorders, including chronic infections (24, 25, 26, 27). Giving that the gamma region is composed predominantly of antibodies of the IgG type, higher concentrations of γ -globulins recorded in cattle affected by bronchopneumonia may reflect inflammation (28). Piccione (29), and Rodrigues (27), indicated that the increases in globulin fractions, particularly γ -globulins are associated with chronic antigenic stimulation, that is revealed in our study by Alb/G ratio highly significant ($p < 0,01$, Table 2). The relative decreased albumin and increase d globulins 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 (28, 11). Moreover, since albumin is a negative acute phase protein, the aforementioned factors probably contributed to significantly lower concentrations of albumin in bovine suffering from bronchopneumonia observed in the present study. The exact demonstration and visualization of individual proteins present in each area of the electrophoretic trace in the study was not performed. but according to previous published data and the results of our study, we can deduct that the high concentration of α globulins zone have

the same meaning as the increase of such acute phase protein like Haptoglobin. Finally, determination of the individual protein changes represents a possible area for future investigations, further studies would be helpful to clarify the effect of respiratory diseases on the concentrations of globulin, which may be helpful in final diagnosis and providing a basis for further specific bovine medical chemistry.

CONCLUSION

In conclusion, the present study suggests that bovine bronchopneumonia may affect the electrophoretic profile of serum proteins with a marked shift. While total protein concentrations, including β -, γ -globulins and especially α_1 -globulins, were significantly higher in cattle with respiratory disease, the albumin and α_2 -globulins values were not significantly increased in the affected bovine. The α -globulin fraction includes many of the acute phase proteins (ceruloplasmin, haptoglobin, α_1 -acid glycoprotein, some lipoproteins), which have great potential as biomarkers of many economically important diseases. Also, the current study showed in cattle suffering from bronchopneumonia markedly higher concentrations of acute phase proteins (haptoglobin) as compared to clinically healthy cattle, which probably influenced the changes recorded in their electrophoretic serum protein pattern. These results confirm the usefulness of SPE measurements in monitoring animals with respiratory diseases. We therefore propose to consider the use of SPE, particularly the α_1 globulins fraction, as an alternative diagnostic contribution to the dosage of acute phase proteins, which remains essential but much more expensive for the research of cattle suffering from bronchopneumonia, subject to further research.

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