Electrophoretic profile of serum protein fractions from sheep naturally infected with Babesia ovis

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SUMMARY

The aim of this study was to investigate the alterations of the serum protein electrophoretic profile in sheep babesiosis. For that, blood sera were sampled from 20 clinically healthy without protozoon Akkaraman sheep, 1.5 to 2.0 years old, stemming from the Van region (Turkey) in one hand and from 36 Akkaraman sheep, naturally infected with B. ovis and with clinical signs before and 5 days after anti-parasitic treatment in the other hand and were analyzed by cellulose acetate electrophoresis. The total proteinemia (P < 0.01) as well as the concentrations of all serum protein fractions (albumin, α1-, β-, γ-globulins and particularly α2- globulins, P < 0.05 to P < 0.001) were dramatically decreased in diseased animals before treatment compared to controls. By contrast, the total protein concentrations and the absolute globulin concentrations significantly increased 5 days after treatment (except for the α1-globulin concentrations) but they remained weakly below the control values. Furthermore, the ratio A/G significantly increased in parallel. These results suggest that B. ovis infection has induced an intense proteolysis of the circulating proteins probably coupled to withdraw of liver protein synthesis and dehydration which was alleviated since parasite eradication.

Keywords: Babesiosis, electrophoresis, serum proteins, sheep, albumin, globulin.

RÉSUMÉ

Profil électrophorétique des protéines sériques des moutons naturellement infectés par Babesia ovis

Le but de cette étude a été de rechercher les altérations du profil électrophorétique des protéines sériques durant la babésiose ovine. Pour cela, les séums de 20 moutons Akkaraman cliniquement sains et indemnes de protozoaire, âgés de 1.5 à 2 ans et issus de la région de Van (Turquie) d’une part et ceux de 36 moutons Akkaraman infectés naturellement par B. ovis et présentant des signes cliniques de babésiose prélevés avant et 5 jours après un traitement antiparasitaire d’autre part, ont été analysés par électrophorèse sur acétate de cellulose. La protéinémie totale (P < 0.01) ainsi que les concentrations de toutes les fractions protéiques (albumine, α1-, β-, γ-globulines et particulièrement les α2- globulines, P < 0.05 à P < 0.001) ont été nettement diminuées chez les animaux malades avant traitement comparé aux valeurs observées chez les contrôles. En revanche, les concentrations en protéines totales et les concentrations absolues en globulines ont significativement augmenté 5 jours après le traitement (à l’exception de celles des α1- globulines) mais elles sont demeurées plus faibles que les valeurs obtenues chez les contrôles. En outre, le ratio A/G a significativement augmenté en parallèle. Ces résultats suggèrent que l’infection par B. ovis a induit une intense protéolyse des protéines circulantes probablement couplée à une diminution des capacités de synthèse hépatique et à un état de déshydratation qui s’atténue dès l’éradication du parasite.

Mots clés : Babésiose, électrophorèse, Protéines sériques, mouton, albumine, globulines.

Introduction

Cases of theileriosis, babesiosis and anaplasmosis, are frequently seen during summer months when ticks are active. Among these diseases, babesiosis, a protozoan disease, is commonly seen in tropical, subtropical and even in temperate regions. Furthermore, this disease affects cattle, goats and sheep and causes great economic losses in Turkey [2, 9, 10, 18].

The normal serum protein electrophoresis leads to identification of 5 main protein fractions composed by albumin, α1, α2, β and γ-globulins respectively. The serum protein electrophoretic profile was altered in various conditions, such as different infectious diseases, liver disorders, acute inflammatory and proliferative cases, tissue damage like trauma, and many other physiological disorders. The albumin value frequently and markedly declines during different diseases, the α-globulin value increases mainly in traumas and some alterations of the lipoprotein metabolism induce changes in the β-globulin fraction. However, since many factors are responsible for these changes, it is generally difficult to identify them [7, 8, 11, 15 - 17].

The serum fraction of pre-albumin, synthesized in liver, declines in patients using oral contraceptives, but also during pregnancy, inflammatory cases, malign tumours, malnutrition and liver diseases. The serum albumin strongly implicated in transport of various lipophilic compounds (bilirubin, long chain fatty acids, iodated thyroid hormones, corticoids, pharmaceutical drugs) and of divalent cations (Ca2+ and Cu2+) can be considered as an endogenous amino-acid store [4]. The main proteins of the α2-globulin fraction, namely α2-macroglobulin and haptoglobin, are affected by ceruloplasmin depletion in the Wilson disease and in malnutrition [4]. The β-globulin fraction is useful for the diagnosis of cancers involving B-lymphocytes and can be used as a fraction test in patients undergoing kidney transplantation [16]. Immunoglobulins

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and the C-reactive protein (CRP) migrate in the γ-globulin area [8, 11, 15, 16].

The aim of this study was to explore the specific effects of the Babesia infection, an important parasite disease, on the different serum protein fractions in sheep.

**Materials and Methods**

**ANIMALS AND BLOOD SAMPLING**

This study was conducted on Akkaraman sheep, 1.5 to 2.0 years old, stemming from Van, Turkey and its surrounding area, naturally infected with babesiosis (n = 36) or healthy (n = 20). Diseased animals exhibited apathy, hyperthermia (40 to 42°C), anaemia, petechial bleeding, icterus and haemoglobinuria as main clinical signs and the babesia infection was confirmed by blood cytological analysis. Smears were prepared from whole blood collected from the *V. auricularis*, stained with 5% Giemsa solution then examined in the immersion objective (X100). *Piroplasms* with a 1.2-1.5 μm diameter, in single / double pear or in other forms were observed in regions close to the erythrocyte periphery. Thereafter, these animals were treated by the intramuscular route with 7% diminazen aceturate solution (Fa. Try. Banil R. T. U., VETAS co. Turkey) at the dose of 3.5 mg/kg and oxytetracyclin (Panox LA, SANOVEL co. Turkey) at the dose of 10-20 mg/kg for *Anaplasma* eradication and with vitamin B12 (Dodex, DEV A co., Turkey, 250 μg in toto, IM, each day for 2 weeks) for stimulating the haematopoiesis. The control sheep were clinically healthy and their haematological and biochemical analyses were normal.

Blood samples were collected by puncture of the *V. jugularis* in all sheep (before and 5 days after the treatment in babesia infected sheep) into sterile tubes without anticoagulant. After clotting for 5 minutes at room temperature (22°C), the serum phase was carefully harvested, centrifuged at room temperature (22°C) for 5 minutes at 500 g and stored at -20°C until analysis.

**SERUM PROTEIN ELECTROPHORESIS**

Basically, total protein concentrations were analysed using the biuret method [20]. The serum protein fractions were separated using the Helena Lab-Titan III® Serum Protein Electrophoresis device (Cat No. 3023), Helena Lab -Titan III Cellulose acetate cards and Electra HR Buffer (Cat No. 5805) tampon solutions (Helena, Bioscience Europe, UK) then stained with Ponceau S Stain solution.

**STATISTICAL ANALYSIS**

The data between control and diseased animals were analyzed with a One Way Variance analysis and the Duncan test was applied for multiple comparisons. Differences were considered as significant when *P* value was less than 0.05.

**Results**

The serum total protein concentrations were significantly depressed in *B. ovis* affected sheep before treatment compared to healthy controls (*P < 0.01*) (Table I). Furthermore, 4.60% of diseased sheep exhibited a serum total protein concentration below 44.80 g/L (Table II). The ratio albumin/globulins was unchanged even if the relative proportions (expressed in %) of some protein fractions were significantly modified in the diseased sheep group before treatment: the mean percentages of the α1- (3.14%) and β-globulins (9.52%) were increased (*P < 0.01* and *P < 0.05* respectively) and the α2-globulin mean proportion (13.26%) was decreased (*P < 0.001*) (Table I). When the respective absolute concentrations were consi-
Serum proteins | Threshold value (g/L) | B. ovis infected sheep
--- | --- | ---
Total proteins | 44.80 | 4.60% | 3.45%
Albumin | 17.88 | 1.83% | 1.29%
α₁-globulin | 0.31 | 0.13% | 0.11%
α₂-globulin | 6.89 | 0.75% | 0.46%
β-globulin | 4.43 | 0.46% | 0.33%
γ-globulin | 18.15 | 1.99% | 1.26%

TABLE II: Frequency (in %) of B. ovis infected sheep (n = 36) whose serum protein fractions (expressed as g/L) were below the corresponding threshold value in healthy controls (n = 20) [defined as mean - 2SEM (standard error)] before treatment and 5 days after.

Discussion

There are different methods for protein analysis to obtain beneficial results in terms of pathogenesis, diagnosis, evolution and prognosis. Various electrophoresis techniques are also used for this purpose. Although protein electrophoresis was extensively used in human medicine since 1950s, these techniques are not used in veterinary medicine as commonly as in human medicine [22]. Many researchers notified that fractions display important differences among all domestic animals between healthy and infected animals [1, 12, 21]. As far as sheep are concerned, values of serum protein fractions in healthy animals differ among previous studies [5, 12, 13]. Moreover, no previous report about alterations of the electrophoretic serum protein profile in sheep with babesiosis was found in the literature.

An increase of the total plasma / serum protein concentrations (hyperproteinemia) is rarely observed and results from water loss during dehydration. This situation is encountered in diseases associated vomiting and diarrhoea causing dehydration, in case of diabetic acidosis, of shock and of some types of neoplasms, such as lymphosarcomas [20]. In literature, we could not found previous report about changes of the electrophoretic serum protein profile in sheep infected with B. ovis, but, there are some articles about other Babesia species.

LOBETTI et al. [14] stated that total protein concentrations decreased in dogs with babesiosis and CAMACHO et al. [6] established that this biochemical parameter was depressed in...
23. - YERUHAM I., AVIDAR Y., AROCH I., HADANI A.: Intra-uterine infection of the concentrations of the total circulating proteins and of all serum fractions in sheep probably because of an intense proteolysis of the circulating proteins coupled to the withdrawal of liver protein synthesis and dehydration. It can be suggested that protozoa remained in the blood of the infected
sheep before the formation of immunity, thereafter disappeared all the more rapidly since a treatment was applied.

References