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Short Paper

Serum protein alterations in goats naturally infected with *Babesia ovis*

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Summary

This study was conducted to determine the electrophoretic pattern of serum protein in goats infected with *Babesia ovis*. Serum total protein was calculated and serum electrophoresis from 15 goats naturally infected with *B. ovis* as well as same number of healthy goats was performed. Microscopic examination of Giemsa-stained peripheral blood smears revealed *B. ovis* infection. The parasitological diagnosis was confirmed using polymerase chain reaction (PCR) assay by using a pair of *B. ovis*-specific primers. Mean values total protein and α and γ globulin fractions in infected goats were significantly higher ($P<0.05$) than those observed in the healthy group.

Key words: Serum protein, Electrophoresis, *Babesia ovis*, Goats, PCR

Introduction

Serum protein electrophoresis is a valuable tool for evaluating health of body, both in diagnosing and clinical monitoring of several diseases (Abate *et al*., 2000). Although a specific diagnosis can rarely be made with serum protein electrophoresis, processes of diseases can be recognized by serum protein patterns, and alterations to these patterns are associated with a variety of different conditions and diseases (Lobetti *et al*., 2000).

Blood serum contains two major proteins including albumin and globulin. Serum protein electrophoresis from healthy goats exhibits four fractions: albumin, α, β and γ fractions, which, in β-globulin was divided to β_1 and β_2 (Alberghina *et al*., 2010).

Recent studies have focused on serum protein alterations in canine babesiosis (Abate *et al*., 2000; Camacho *et al*., 2005); however, there are no data regarding serum protein pattern in goats naturally infected with *B. ovis.* Therefore, this study was conducted to evaluate the specific effects of *Babesia* infection on different serum protein fractions in goats, as well as to assess its application in diagnosis by study of the quantitative alterations in serum proteins associated to the disease.

Materials and Methods

Thirty goats of different local breed (West-Azerbaijan) were studied and classified into two groups. All of the animals were more than 1-year-old (≥1 year). The infected group was comprised of 15 goats (10 female and 5 male) naturally affected by *B. ovis.* As control group, 15 clinically healthy goats (8 female and 7 male) were also sampled from the same farm. Jugular

blood samples were obtained from infected and control goats. Blood serum was then separated by centrifugation at 750 g for 15 min and stored at -20°C until measurements were done. Also, blood samples from ear vein were obtained for the preparation of thin blood smears, stained with Giemsa, for the detection of *Babesia* piroplasms. PCR was performed not only to detect *B. ovis* in blood of infected animals but also to rule out the presence of *Babesia* spp. infection in both healthy and infected animals. DNA was extracted using a DNA purification kit (Fermentas, Germany) according to the manufacturer's instructions. One pair of primers, P1 (5'-CACAGGGAGGTAGTGACAAG-3') and P2 (5'-AAGAATTTCACCTATGACAG-3'), specific for amplification of *Babesia* species 18S ssu RNA (Shayan *et al*., 2007), were used in PCR assay as a positive quality control for the *Babesia* species. Seminested PCR of the PCR products of *B. crassa* with primer pair of P2 (5'-AAGAATTTCACCTATGACAG-3') and P4 (5'-GTTATGGCCCGTTGGCTTAT-3') and *B. motasi* with primers P2 (5'-AAGAATTTCACCTATGACAG-3') and P5 (5'-CGCGATTCCGTTATTGGAG-3') were conducted as well (Shayan *et al*., 2007). In addition, amplification of *B. ovis* was performed by species-specific primer pair previously reported and used to amplify a fragment of 549 bp. The sequences of primers were as follows:

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Bbo-F, 5'-TGGGCAGGACCTTGGTTGTTCT-3'
Bbo-R, 5'-CCGCGTAGCGCCGGCTAAATA-3'
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PCR was performed in 50 µl total reaction volume containing 5 µl of 10 × PCR buffer, 2 mM $MgCl_2$, 250 µM of each of the four deoxynucleotide triphosphate, 1.25 U Taq DNA polymerase (Fermentas, Germany), 50 pmol of each primer and 50 ng of extracted DNA. For negative control, distilled water was added instead of nucleic acid. PCR reaction was performed in a DNA thermocycler (Model CP2-003; Corbett Research, Australia). Cycling condition was 95°C for 5 min, followed by 45 cycles at 94°C for 45 sec, 63°C for 45 sec and 72°C for 1 min with a final extension step of 72°C for 10 min. Amplified products were electrophoresed on 2% agarose gel and stained with ethidium bromide to visualize the amplified DNA fragments under ultraviolet light. Serum total protein concentrations were determined by Biuret method and spectrophotometer (Perkin-Elimer, Junior model 35). Albumin and globulins were separated by cellulose acetate (Helena, France) electrophoresis in barbital buffer (Helena, France), pH = 8.6 at 180 V, 4 mA, for 15 min using Sebia system (Sebia, France). The separated proteins were stained with Panceau S (Sigma, Germany), and then destained. After plate drying at 50-60°C, the relative levels of separated proteins were scanned by densitometer at 525 nm. Data was checked graphically (histograms, q-q plots and box plots) for the outliers and normality. No extreme outlier was detected and the assumptions were satisfied. Independent samples t-test was used to compare the differences between groups. The level of significance was set at $P<0.05$. Statistical analyses were carried out using SPSS (Release 17, SPSS Inc., and Chicago, Illinois). Results are presented as means±SEM.

Results

All of the infected animals were positive by PCR with *B. ovis*-specific primers (549 bp). On the contrary, there was no amplification of *B. ovis* DNA in the control animals (Fig. 1).

Serum protein electrophoresis of samples exhibited four fractions: albumin, α, β and γ fractions in goats which, in the samples, α globulin band was divided into α_1 and α_2. Electrophoretic pattern of serum from healthy and infected goats is shown in Fig. 2.

Concentrations (mean±SEM) of serum total protein, albumin and globulins of healthy and affected animals are presented in Table 1.

Discussion

Although evaluation of serum proteins and their electrophoretic profiles have been widely used in human medicine since the 1950s, in veterinary medicine the mentioned technique is not used as a common diagnostic method for the evaluation of

dysproteinemias (Attaelmannan and Levinson, 2000). Many researchers stated that fractions display important differences among all domestic animals between healthy and infected animals (Kean and Doxey, 1981). As far as goats are concerned, values of serum protein fractions in healthy animals disagreed with a previous study (Mendes Ahid and Soto-Blanco, 2010).

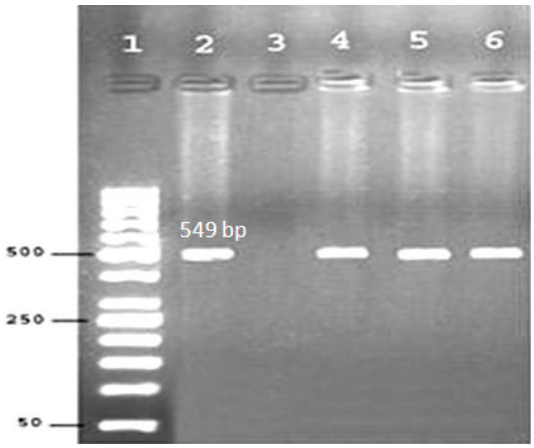

Fig. 1: PCR products amplified using *B. ovis* specific primers. Lane 1: 50 bp molecular ladder, Lane 2: positive blood sample (positive control), Lane 3: distilled water (negative control), and Lane 4-6: PCR products from infected goats

Earlier studies showed that protein fractions display important differences among all domestic animals between healthy and *Babesia*-infected animals (Barrera *et al*., 2010).

In our study, the mean value obtained for serum total protein in the affected animals was significantly higher (P<0.05) than that obtained in the healthy ones. Hyperproteinemia in babesiosis is usually caused by dehydration, resulting from lethargy and anorexia associated with the disease (Divers, 1998). The hydration leads to hemconcentration through reduction in fluid volume and consequent hyper-proteinemia (Kaneko *et al*., 2008).

Table 1: Concentration (means±SEM) of serum total protein and protein fractions (g/dl) in healthy and infected goats

Parameter	Control (n=15)	Infected (n=15)
Tp†	7.05 ± 0.01	7.50 ± 0.09*
Alb‡	2.70 ± 0.06	2.22 ± 0.01**
α_1	0.85 ± 0.02	1.31 ± 0.07**
α_2	1.00 ± 0.03	1.04 ± 0.02NS
β	0.58 ± 0.02	0.63 ± 0.01NS
γ	1.90 ± 0.03	2.20 ± 0.04**

Asterisks indicate the presence of the significance between the two groups, * P<0.01 and ** P<0.05. NS: Non-statistically significant. *Tp*†: Total protein, and *Alb*‡: Albumin

The mean concentration of albumin in healthy animals was significantly higher (P<0.05) than that obtained in diseased animals. The hypoalbuminemia observed in goats in this study is in agreement with the findings of Apaydin and Dede (2010) and Esmaeilnejad *et al*. (2012) in ovine babesiosis. The most likely explanation for this finding would be hepatopathy that may develop during the disease (Colville, 2002), to the symptomatic anorexia state (Kaneko *et al*., 2008) and urinary loss of albumin associated with renal failure (Camacho *et*

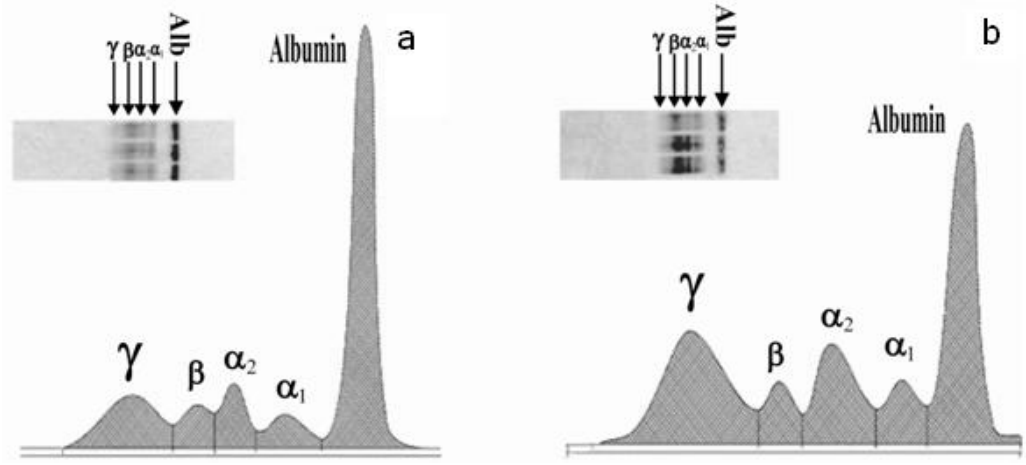

Fig. 2: Representative serum protein patterns of healthy (a) and infected (b) goats

al., 2005).

The proteins located in α_1-globulin band are significantly increased ($P<0.05$) in diseased animals compared with the healthy ones. These results are in accordance with the findings of Camacho *et al*. (2005). The most likely reason for this elevation is the release of acute phase reactants, such as α_1-antitrypsin, α_1-acid glycoprotein, α_2-macroglobulin and haptoglobin in response to inflammation process (Barrera *et al*., 2010).

When the result of β-globulin sub-fraction in goats with babesiosis was compared with those obtained in the control group, no significant difference was observed (Table 1). An increase in its concentration related to acute hepatic damage has been described as a consequence of the increase in transferrin concentration (Kaneko *et al*., 2008). This protein also contributes to the β-globulin decrease if the hepatic disease is chronic (Barrera *et al*., 2010). Increase in the plasminogen concentration, which is included in the β-globulin fraction, due to intra-vascular coagulation may be explained by elevation of β-globulin concentration (Kaneko *et al*., 2008). However, further studies are needed to precisely assess the acute phase response in caprine babesiosis. Finally, concentration of β-globulin fraction may be related to the albumin fraction in cases of extra-vascular liquid exudation (Barrera *et al*., 2010). This fact, namely hypoalbuminemia and lower β-globulin was observed in present study.

The immunity mechanisms in babesiosis are mainly of humoral nature (Rahbari *et al*., 2008), although the protozoa can activate a cellular-type immune response (Tizard, 1996). In accordance with the findings of other studies (Camacho *et al*., 2005), our results indicated that the mean value obtained for the γ-globulin fraction in infected goats was significantly higher ($P<0.05$) than the one obtained in healthy goats. This increase, observed in the serum γ-globulins concentration is related to the humoral immunity activation by parasitation of the animals studied (Barrera *et al*., 2010).

The present study revealed that the increase of total protein, associated with the increase of α and β globulin fractions, is a common finding in serum electrophoresis of goats suffering from babesiosis, which can be useful for diagnosis and prognosis of caprine babesiosis as a supplementary analysis in combination with clinical, parasitological and molecular findings of this disease.

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