Turkish Journal of Veterinary & Animal Sciences

Volume 36 | Number 1 Article 2

1-1-2012

Serum protein fractions in Brazilian-breed donkeys using agarose gel electrophoresis

PAULO HENRIQUE CAVALCANTE

ANDREA CRISTINA SILVA

SIDNEI SAKAMOTO

BENITO SOTO-BLANCO

TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2012; 36(1): 9-12
© TÜBİTAK
doi:10.3906/vet-1011-565

Serum protein fractions in Brazilian-breed donkeys using agarose gel electrophoresis

Paulo Henrique CAVALCANTE, Andréa Cristina C. SILVA, Sidnei M. SAKAMOTO, Benito SOTO BLANCO*

Department of Animal Sciences, Universidade Federal Rural do Semi-Árido (UFERSA), BR 110 Km 47, Mossoró, RN 59625-900 - BRAZIL

Received: 08.11.2010

Abstract: The values for the serum protein profiles of horses are available in many studies. However, only one study has characterized the electrophoretic migration profile of plasma protein in donkeys. The present study aimed to determine by agarose gel electrophoresis the normal values for serum protein fractions in erratic Brazilian-breed donkeys and to compare these values with those of the serum proteins of horses. Serum samples were collected from 26 donkeys of the Brazilian-breed and from 10 healthy horses. The serum levels of the total proteins were determined by the biuret method. The separation and determination of the values of serum protein fractions were performed by means of agarose gel electrophoresis and the analysis of the data was done using specific software. The results were compared using an unpaired t-test, with the level of statistical significance set at $P < 0.05$. A total of 7 protein fractions were found: albumin and α1-, α2-, β1-, β2-, γ1-, and γ2-globulins. The results showed that in the evaluated donkeys the levels of α2-globulins were higher than those of α1-globulins and that the levels of γ1-globulins were higher than those of γ2-globulins. Furthermore, the sexual interference in the concentrations of proteins and their fractions revealed that no sex-related difference was present. The serum concentrations of protein fractions from the donkeys were similar to those of horses. Sex did not affect the serum protein levels.

Key words: Albumin, donkeys, globulins, proteinogram, proteins

Introduction

The determination of plasma protein profiles is a technique of laboratory diagnosis that is helpful in differentiating the cause of dysproteinemia. The technique is used in the differentiation of acute from chronic disease states and in the diagnosis of inflammation. Electrophoresis is the technique that is used as a reference method for the fractionation and quantification of serum proteins in clinical biochemistry. The principles of electrophoresis are based on the knowledge of the chemical composition of proteins and factors (charge and molecular weight) that determine their electrophoretic migration (1,2).

The values for the serum protein profiles of horses are available in many studies (3-5); however, only one study has characterized the electrophoretic migration profile of plasma protein in donkeys (6). Knowledge of the values of the serum protein electrophoresis profile in donkeys will certainly contribute to a better interpretation of the results with regard to the species. This study aimed to determine, by agarose gel electrophoresis, the reference values for the serum protein fractions of free-living Brazilian-breed donkeys and to compare these values with those of the serum proteins of horses.

* E-mail: soto-blanco@ufersa.edu.br

Materials and methods

We used 26 donkeys (14 males and 12 females) from the Brazilian-breed that were erratic (freely living) in the western region of Rio Grande do Norte state, Brazil. We also used 10 horses (4 males and 6 females) from properties in the city of Mossoró, RN, Brazil. Blood samples were collected by jugular venipuncture, into plain tubes. Sera were used for the determination of total protein by colorimetric assay, and protein fractions were separated by electrophoresis. Protein analysis was performed by the biuret method in an automated biochemistry analyzer (SBA 200, CELM, Barueri, SP, Brazil) using a commercially available test (KATAL, Belo Horizonte, MG, Brazil).

Agarose gel electrophoresis was performed with agarose kits (CELM, Barueri, SP, Brazil) and with the SE-250 electrophoresis system (CELM). After protein separation at pH 8.5, the gels were stained with Amido Black solution and then scanned and analyzed with a densitometer (DensitScan version 2.2, CELM). The points of separation between fractions on the resultant serum protein electrophoresis (SPE) profiles were marked manually, using the midpoint between peaks on the electrophoretogram. The protein concentrations (g/dL) were determined by multiplying the percentage of each fraction by the total protein concentration. Total globulins were calculated as the sum of the non-albumin protein concentrations. The protein fractions that were determined included albumin and α1-, α2-, β1-, β2-, γ1-, and γ2-globulins. The albumin/globulin (A/G) ratio was calculated manually.

The data are presented as averages followed by their respective standard deviations. Statistical analysis was performed using the Graph Pad InStat version 3.01 statistical software package. The results were compared using an unpaired t-test or analysis of variance (ANOVA) plus a post hoc Bonferroni test. The level of statistical significance was set at $P < 0.05$.

Results

The donkey and horse serum total proteins and their fractions, separated by electrophoresis, are presented in Table 1. A total of 7 protein fractions were obtained in both the donkeys and horses: albumin and α1-, α2-, β1-, β2-, γ1-, and γ2-globulins. Statistical comparison of the values of both species showed no significant difference ($P < 0.05$).

Table 1. Serum proteins from Brazilian-breed donkeys and from horses. Data are presented as mean followed by standard deviation.

		Donkeys (n = 26)	Horses (n = 10)
Total Proteins (g/dL)		7.44 ± 0.68	7.37 ± 0.56
Albumin	%	37.7 ± 5.79	39.2 ± 6.61
	g/dL	2.93 ± 0.47	2.76 ± 0.51
Globulins	%	62.3 ± 5.79	60.8 ± 6.61
	g/dL	4.46 ± 0.70	4.65 ± 0.58
α1-globulins	%	6.53 ± 1.73	6.26 ± 2.17
	g/dL	0.48 ± 0.19	0.48 ± 0.16
α2-globulins	%	9.26 ± 2.48	10.1 ± 2.61
	g/dL	0.75 ± 0.23	0.64 ± 0.16
β1-globulins	%	10.7 ± 3.09	11.0 ± 6.56
	g/dL	0.79 ± 0.54	0.77 ± 0.24
β2-globulins	%	10.0 ± 4.58	10.2 ± 2.82
	g/dL	0.76 ± 0.23	0.76 ± 0.35
γ1-globulins	%	19.3 ± 4.64	17.2 ± 4.31
	g/dL	1.25 ± 0.31	1.50 ± 0.35
γ2-globulins	%	6.70 ± 1.67	6.06 ± 1.40
	g/dL	0.44 ± 0.09	0.52 ± 0.13
A/G ratio		0.62 ± 0.15	0.66 ± 0.19

$P > 0.05$, unpaired t-test

The percentages of the fractions of serum proteins, separated by electrophoresis, on male and female donkeys are presented in Table 2. There were no significant differences ($P < 0.05$) between males and females.

Discussion

The values for the serum protein profiles of horses are widely available in the experimental literature (3-5), but there is just one study of donkeys (6). The levels found in the horses in our study were close to the values presented by other authors (3-5). The minor variations in the values found here, compared with those from the literature, are probably due to differences in technique and to genetic, geographical, and nutritional differences.

Table 2. Serum proteins fractions (in %) from male and female Brazilian-breed donkeys and horses. Data are presented as mean followed by standard deviation.

	Male donkeys (n = 14)	Female donkeys (n = 12)	Male horses (n = 4)	Female horses (n = 6)
Albumin	38.6 ± 6.73	36.6 ± 4.34	40.6 ± 9.16	38.2 ± 4.60
α1-globulins	6.45 ± 1.93	6.64 ± 1.53	4.78 ± 0.81	7.44 ± 2.24
α2-globulins	9.28 ± 2.22	9.23 ± 2.90	9.70 ± 3.40	10.4 ± 2.16
β1-globulins	11.1 ± 3.06	10.2 ± 3.20	11.6 ± 7.94	10.5 ± 6.19
β2-globulins	9.32 ± 4.51	11.2 ± 4.56	11.7 ± 3.41	9.02 ± 1.79
γ1-globulins	18.7 ± 4.81	20.2 ± 4.52	16.1 ± 3.45	18.1 ± 5.10
γ2-globulins	6.98 ± 1.79	6.32 ± 1.52	5.60 ± 1.93	6.42 ± 0.88

P > 0.05, analysis of variance (ANOVA) plus a post hoc Bonferroni test

The values of the total serum proteins found for donkeys in the present study were slightly higher than those found in donkeys of the Brazilian-breed (7) and in donkeys of the Basse-Normandie breed (8), but were similar to those of UK donkeys (9). Albumin serum levels in our study were within the ranges presented for USA donkeys (10), UK donkeys (9), Brazilian donkeys (7), and Basse-Normandie donkeys (8), but were lower than those of Poitou and Indian donkeys (11).

We found 7 protein fractions in the donkeys: albumin and α1-, α2-, β1-, β2-, γ1-, and γ2-globulins. The same fractions were found in the horses. In Ragusana donkeys, 5 protein fractions were identified: albumin and α1-, α2-, β-, and γ-globulins (6). The probable reason for the variation is a difference in technique; agarose gel electrophoresis was used in the present study, and capillary electrophoresis was used in the study of Ragusana donkeys (6).

The Brazilian donkeys from our study presented lower percentages of α2- and γ-globulins and higher percentages of β-globulins than Ragusana donkeys (6). The divergences in these values are likely to be due to differences between techniques and to genetic and geographical nutritional differences.

We found the levels of α2-globulins to be higher than that of α1-globulins in both horses and donkeys, data similar to those observed in Ragusana donkeys (6). There are no known functional differences between the 2 fractions (3). The fractions of α-globulins are associated with acute phase proteins, and increased levels of these fractions are associated with acute inflammatory processes (3).

In our study, the concentration of γ1-globulins was higher than that of γ2-globulins in both donkeys and horses. In pathological conditions, there is some increase in serum globulin as a result of increased production, especially in inflammatory processes. An increased concentration of γ-globulins is usually associated with the improved production of antibodies, and chronic inflammatory processes are usually responsible for increases in the fractions of β- and γ-globulins (1,2). The reduction in the concentration of serum globulins (especially that of γ-globulins) is related mainly to the non-transfer of passive immunity in young animals (12-14), but other acquired causes and primary genetic immunodeficiencies may occur (15,16).

Sex is considered to be an important factor that can change the parameters of hematological and serum biochemistry in animals, including donkeys (8,17). In this study, the sexual interference in the concentrations of proteins and their fractions was not verified. In fact, both testosterone and estrogen present an anabolic effect (2), which is the reason for the absence of sex interference on serum protein levels.

In conclusion, this study provides values for serum protein fractions in erratic Brazilian-breed donkeys, through the use of agarose gel electrophoresis. The serum protein profile of Brazilian-breed donkeys is similar to that of horses. The separation of serum protein fractions by agarose gel electrophoresis revealed 7 protein fractions. The levels of α-2 globulins were higher than those of α-1 globulins, and the concentration of γ1-globulins was higher than that of γ2-globulins. Sex did not affect the serum protein levels.

References

1. Thomas, J.S.: Protein electrophoresis. In: Feldman, B.F., Zinkl, J.G., Jain, N.C., Eds. Schalm's Veterinary Hematology. 5th ed., Blackwell Publ., Ames, 2006; 899-903.
2. Eckersall, P.D.: Proteins, proteomics, and the dysproteinemias. In: Kaneko, J.J., Harvey, J.W., Bruss, M.L., Eds. Clinical Biochemistry of Domestic Animals. 6th ed., Academic Press, Burlington. 2008; 117-155.
3. Carapeto, M.V., Barrera, R., Mañe, M.C., Zaragoza, C.: Serum α-globulin fraction in horses is related to changes in the acute phase proteins. J. Equine Vet. Sci., 2006; 26: 120-127.
4. Agrícola, R., Carvalho, H., Barbosa, M., Pereira, M., Medeiros, J.A.S., Ferreira-Dias, G.: Blood lymphocyte subpopulations, neutrophil phagocytosis and proteinogram during late pregnancy and postpartum in mares. Reprod. Dom. An., 2008; 43: 212-217.
5. Kaneko, J.J., Harvey, J.W., Bruss, M.L.: Clinical Biochemistry of Domestic Animals. 6 th ed., Academic Press, Burlington. 2008; 908-915.
6. Caldin, M., Furlanello, T., Solano-Gallego, L., Lorenzi, D.D., Carli, E., Tasca, S., Lubas, G.: Reference ranges for haematology, biochemical profile and electrophoresis in a single herd of Ragusana donkeys from Sicily (Italy). Comp. Clin. Path., 2005; 14: 5-12.
7. Mori, E., Fernandes, W.R., Mirandola, R.M.S., Kubo, G., Ferreira, R.R., Oliveira, J.V., Gacek, F.: Reference values on serum biochemical parameters of Brazilian donkey (*Equus asinus*) breed. J. Equine Vet. Sci., 2003; 23: 358-364.
8. Pitel, P., Moulin, M., Valette, J.P., Dumontier, S., Petit, L., Fortier, G., Couroucé-Malblanc, A.: Approach of hematological and blood biochemistry values in two breeds of donkeys. Pratique Vétérinaire Équine, 2006; 38(149): 19-25. (article in French with an abstract in English).
9. French, J.M., Patrick, V.H.: Reference values for physiological, haematological and biochemical parameters in domestic donkeys (*Equus asinus*). Eq. Vet. Educ., 1995; 7, 33-35.
10. Zinkl, J.G., Mae, D., Guzman, P., Farver, T.B., Humble, J.A.: Reference ranges and the influence of age and sex on hematologic and serum biochemical values in donkeys (*Equus minus*). Am. J. Vet. Res., 1990; 51: 408-413.
11. Gupta, A.K., Varshney, I.P., Uppal, P.K.: Comparative studies on biochemical indices in different breeds of equines. Indian Vet. J., 1994; 71: 26-30.
12. Morris, D.D., Meirs, D.A., Merryman, G.S.: Passive transfer in horses: incidence and causative factors on a breeding farm. J. Am. Vet. Res., 1985; 46: 2294-2299.
13. Lavoire, J.P., Spensley, M.S., Smith, B.P., Mihalyi, J.: Absorption of bovine colostral immunoglobulins G and M in newborn foals. Am. J. Vet. Res., 1989; 50: 1598-1603.
14. Raidal, S.L., McTaggart, C., Penhale, J.: Effect of withholding macromolecules on the duration of intestinal permeability to colostral IgG in foals. Aust. Vet. J., 2005; 83: 78-81.
15. Prryman, L.E.: Primary and secondary immune deficiencies of domestic animals. Adv. Vet. Sci. Comp. Med., 1979; 23: 23-52.
16. Crisman, M.V., Scarratt, W.K.: Immunodeficiency disorders in horses. Vet. Clin. North Am. Equine Pract., 2008; 24: 299-310.
17. Mori, E., Mirandola, R.M.S., Ferreira, R.R., Oliveira, J.V., Gacek, F., Fernandes, W.R.: Reference values on hematologic parameters of the Brazilian donkey (*Equus asinus*) breed. J. Equine Vet. Sci., 2004; 24: 271-276.