Journal of Equine Veterinary Science 33 (2013) 433-436

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Reference Intervals of Serum Protein Concentrations from Clinically Healthy Female Ragusana Donkeys (*Equus asinus*) Determined by Cellulose Acetate Electrophoresis

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ARTICLE INFO

Article history:
Received 10 May 2012
Received in revised form
12 July 2012
Accepted 24 July 2012
Available online 17 November 2012

Keywords:
Ragusana donkey
Serum proteins
Reference intervals
Acetate cellulose electrophoresis

ABSTRACT

The aim of this study was to evaluate total serum protein concentration measured using biuret reaction and the protein fractions determined using acetate cellulose electrophoresis in Ragusana donkeys (*Equus asinus*). Blood samples were collected from 68 clinically healthy female donkeys by jugular venipuncture. The serum levels of total proteins were determined using biuret method, and the separation of proteins was performed using acetate cellulose electrophoresis. Coefficients of variation were also calculated for within-assay precision, and were found to be less than 5% for α- and β_1-globulins and 8% or less for albumin, β_2-, and γ-globulins. A total of five protein fractions were separated and quantified: albumin, α-, β_1-, β_2-, and γ-globulins. Data obtained from young and adult subjects were compared using the Mann–Whitney *U* test. Reference intervals (2.5%-97.5% quantiles) were determined for total proteins (50.0-84.0 g/L), albumin (16.2-36.6 g/L), α-globulins (4.85-19.5 g/L), β_1-globulins (2.25-10.35 g/L), β_2-globulins (3.30-14.85 g/L), γ-globulins (10.0-30.5 g/L), and albumin/globulin ratio (0.41-1.13). In relation to age, statistically significant differences were found in total protein concentration and γ-globulins. The results obtained in the present study contributed to establish reference intervals of serum protein fractions obtained using acetate cellulose electrophoresis in female Ragusana donkeys to be used by practitioners for health control.

1. Introduction

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 that determine their electrophoretic migration [1-3]. Each fraction of serum total proteins is represented by various elements with specific function. Albumin is the main protein of mammal serum, and it is essential for the regulation and keeping of oncotic pressure or osmotic pressure necessary for the proper distribution of body fluids in the vascular compartment and in tissues [4]. Globulins, a heterogeneous group of proteins, are divided into α-, β-, and γ-globulins on the basis of their relative electrophoretic mobilities [5,6]. As variations in albumin and globulin concentrations are

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http://dx.doi.org/10.1016/j.jevs.2012.07.012

index of a pathologic condition, their evaluation using serum protein electrophoresis is a valuable diagnostic tool. Serum protein fractions have been studied in donkeys using agarose gel electrophoresis [3] in a restricted number of subjects [7], but, differently from other species, reference values using acetate cellulose electrophoresis have not been determined in donkeys. As the changes of serum proteins depend on the electrophoretic support method used [8-10], and factors such as aging significantly influence these values [11-13], the aim of the current study was to determine the reference intervals for total proteins, their fractions, and albumin/globulins (A/G) ratio in young and adult donkeys using acetate cellulose automated electrophoresis. For this purpose, we studied the pattern of serum protein fractions in Ragusana donkeys to provide reference values for this breed considering that reference intervals of serum protein fractions can also be influenced by breed in some species [14].

2. Materials and Methods

Most of the farms with Ragusana donkeys are in the Sicily (latitude 38°6′43”56N, longitude 13°20′11”76E) region. Recently, the interest for donkey has increased for asinine milk production, well tolerated by human infants with an allergy to cow's milk. A total of 68 female donkeys, aged between 4 months and 12 years and belonging to the same farm, were divided into two groups: young and adult donkeys. Thirty young donkeys (age, 4-24 months; mean age, 14 ± 7 months) and 38 adult donkeys (age, 3-12 years; mean age, 6.5 ± 2.9 years) were used. Before starting the study, donkeys were subjected to a complete clinical examination, including changes in rectal temperature, respiratory and heart rates, and hematological and hematochemical profiles, and all results were considered within normal limits. Animals were free from internal and external parasites. All housing and care conformed to the standard recommended by the Guide for the Care and Use of Laboratory Animals and Directive 86/609 CEE.

For all donkeys, samples were collected by jugular venipuncture always at the same time (8.30 AM-10.30 AM) to avoid the influence of a different photoperiod and ambient temperature on the daily variations of studied parameters, as previously observed in horses [15]. Blood was collected into ethylenediaminetetraacetic acid tubes for hematological studies and into Vacutainer tubes without anticoagulant (Terumo Corporation, Tokyo, Japan) for serum protein measurements. Samples were allowed to clot at room temperature (20°C) and centrifuged at 2081*g* for 15 minutes to separate serum. The serum samples were not lipemic and were not hemolyzed. They were dispensed into plastic tubes and stored at −20°C until analysis. Serum protein electrophoresis was performed within 1 week after sampling.

At the time of analysis, serum samples were thawed at 20°C for 30 minutes before determining protein concentrations. The concentration of serum total proteins was determined by biuret method using an automated analyzer UV Spectrophotometer (SEAC, Slim, Florence, Italy). The protein fractions were performed using an automated system on cellulose acetate films (SelVet 24, SELEO Engineering, Naples, Italy) according to a procedure described in detail elsewhere [12]. Using the computer software SelVet (SELEO Engineering), electrophoresis curves plus related quantitative specific protein concentrations were displayed. All samples were analyzed by the same person. Within-assay precision was tested by performing 12 runs on one asinine serum sample.

Statistical analysis was performed on total serum protein concentrations obtained using the biuret method and protein fractions obtained using electrophoresis. For total proteins, protein fractions and A/G ratio descriptive statistics are provided. Reference intervals were calculated using 97.5%, 50%, and 2.5% quantiles. The D'Agostino−Pearson normality test was used to assess normality. Outliers were identified according to Grubb's test. Coefficient of variation was calculated for within-assay precision of electrophoresis by cellulose acetate and expressed as percentage. A Mann−Whitney *U* test was used to compare the concentrations of total proteins, albumin, globulin fractions, and A/G ratio between young and adult donkeys. A P value $<.05$ was considered statistically significant. All results were expressed as mean ± standard error of the means (standard deviation). The data were analyzed using Statistica 7.5 software package (Statsoft Inc., Tulsa, OK).

3. Results

The total protein concentrations ranged from 50.0 to 84.0 g/L (median 67.9 g/L). Adult donkeys showed levels significantly higher ($P < .05$) than young donkeys (69.0 ± 8.56 g/L and 64.1 ± 9.31, respectively). In all sera analyzed, albumin, α-, β_1-, β_2-, and γ-globulins were resolved (Fig. 1). Significant *P* values (D'Agostino−Pearson test) were found for α-, β_1-, and β_2-globulins, indicating that these results were not normally distributed in this population. One outlier was found for α-globulins, but it was not excluded from the calculation of reference interval because it was close to the 95% confidence intervals. Reference intervals for 68 female donkeys were calculated for the absolute and relative values of each serum protein fraction (Table 1). No significant differences were found between young and

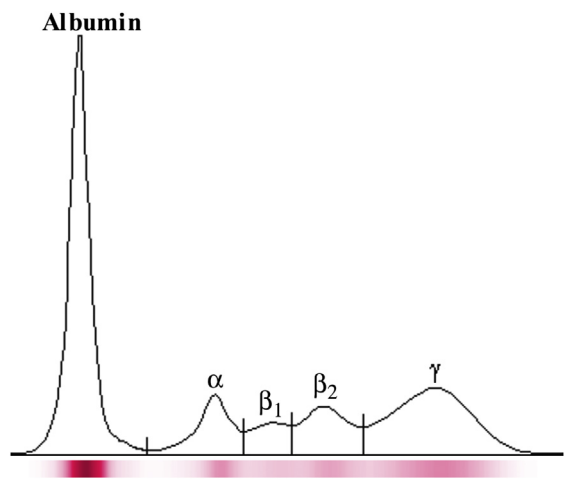

Fig. 1. Representative serum protein electrophoretogram observed in a Ragusana donkey.