Theriogenology xxx (2014) 1–10

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Blood analysis in newborn donkeys: hematology, biochemistry, and blood gases analysis

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ARTICLE INFO

Article history:
Received 19 February 2014
Received in revised form 9 April 2014
Accepted 9 April 2014

Keywords:
Donkey
Biochemistry
Blood gas analysis
Hematology
Newborn

ABSTRACT

The knowledge of reference ranges for hematologic, biochemical, and blood gas parameters in the different species and the influence of breed and age on them is a fundamental tool for the clinician. For this reason, the aim of this study was to evaluate the age-related changes of hematologic and biochemical parameters in Martina Franca donkey foals during the first 3 weeks of life and of blood gases during the first 24 hours of age. Fifteen healthy donkey foals were enrolled; blood samples were collected from each foal at 10 minutes after birth, 1 hour after the first and second suckles, 12 and 24 hours after birth, daily from Day 2 to 7, and at Days 10, 14, and 21 of life. Erythrocytes, leukocytes, and platelets counts were assessed; also metabolic (alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, creatinphospokinase, lactate dehydrogenase, alkaline phosphatase, glucose, blood urea nitrogen, creatinine, total proteins, albumins, cholesterol, and total bilirubin) and electrolytic parameters (Ca, P, Mg, Na, K, and Cl) were evaluated. Finally, blood gases and metabolic parameters (pH, pCO_2, pO_2, sO_2, TCO_2, HCO_3, lactate, and base excess) on venous blood were assessed with a portable analyzer. A statistical analysis to evaluate the influence of age and sex was performed. Several differences were found between sampling times, demonstrating that age influences these parameters. Moreover differences were found compared with data reported in literature for donkey foals of another species, horse foals, and adult donkeys. Although a great interindividual variation for some parameters exists, this study demonstrated that interval references should be addressed not only to different species, but also to specific breeds and to the neonatal period.

1. Introduction

Hematologic and biochemical parameters vary in relation to species, breed, age, and sex. The knowledge of reference values in healthy animals gives a guide for the clinician in evaluating parameters thus allowing to use hematology, biochemistry, and blood gases analysis as important aids in preventive medicine and the diagnosis of diseases. However, the lack of information on reference ranges for particular breeds, environmental conditions, and management systems limits the usefulness of these investigations in donkeys [1,2]. Moreover, age has a major influence on hematologic parameters [3].

Hematologic values in donkeys often are compared with that of horses, but some differences exists. Commonly reticulocytes are not found in donkey peripheral circulation [4,5]; donkeys often present fewer but larger erythrocytes, and a greater mean corpuscular volume (MCV) [5]. Plasma triglyceride levels are usually higher in donkeys compared with horses, whereas bilirubin concentration is lower [5];

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0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.04.004

serum creatine kinase and gamma glutamyl transferase (γGT) activities are higher in donkeys than in horses [5].

Hematologic and biochemical parameters change during the first weeks of life, and several studies have been performed in horse foals [6–11]. To authors' knowledge, only one study examined some hematologic and clinical chemical parameters in donkey foals, and it was performed on Amiata donkeys [12].

Another important tool for daily clinical practice is represented by blood gas analysis, even if the need of arterial blood limits the routine use. In the last year, the use of handheld portable clinical analyzer allowed the evaluation of blood chemical and electrolyte parameters together with pH and gases on venous blood samples. This instrument has been validated in human medicine [13], and it has been used with valid results also in veterinary medicine in the last years [14–17]. No studies on the usefulness of this instrument in adult or newborn donkeys have been actually performed.

For all these reasons, there is a need of specific reference ranges for hematologic, biochemical, and blood gases parameters for the Martina Franca donkey, an Italian breed considered as endangered and on which several studies have been performed in the last years [18–20].

In particular, the aims of this study were to evaluate the age-related changes of hematologic and biochemical parameters in Martina Franca donkey foals during the first 3 weeks of life; moreover, blood gases were assessed during the first 24 hours of age on venous blood.

2. Materials and methods

2.1. Animals

The study was conducted during the breeding season 2012. Fifteen Martina Franca donkey (*Equus asinus*) foals were enrolled, born by spontaneous delivery and housed in the Veterinary Teaching Farm of the University of Teramo.

All foalings were observed, but jennies were allowed to give birth undisturbed and spontaneously. For each jenny, the following characteristics were recorded: age, gestational length, time and type of foaling, and the foal and placental expulsion time. Foal expulsion time was considered to be the interval between the rupture of the allantoic sac and the complete expulsion of the foal. Placental retention was defined as no expulsion of the fetal membranes within 3 hours of foaling [21,22].

All of the donkey foals were at term, with normal birth weight, coat, and fetlock join extension [23]. Some characteristics, such as the activity, pulse, grimace, appearance of the mucous membranes, and respiration index within 10 minutes of birth, the presence of suck and righting reflexes, the time to stand up and to the first suck, and some physical and behavioral characteristics were used to asses foal maturity and viability and were within the normal ranges reported for the horse foal [23,24], and previously used also for the donkey foals [20]. The activity, pulse, grimace, appearance of the mucous membranes, and respiration score system used gives a score of 2, 1, or 0 in relation to the characteristics of heart rate and rhythm (>60 bpm and regular rhythm, irregular rhythm or <60 bpm; absent rhythm), respiratory rhythm (regular, irregular, or absent), body tone (sternal/active, hypotonic, or atonic), and response to stimuli (avoidance of stimulation, grimace/weak, or absent response).

2.2. Experimental protocol

An indwelling catheter (BD Secalon-T 18G) was placed into the left jugular vein of each foal. Blood samples were collected using a 10-mL syringe for hematology and clinical biochemistry and divided into tubes with EDTA for hematology and tubes without anticoagulant for biochemistry. For the blood gases analysis, samples were collected with a 1-mL syringe and directly loaded in the cartridge.

Blood samples were collected from each foal with the following schedule:

- Sample 1: 10 minutes from birth, for all the analyses.
- Sample 2: 1 hour after first suckle, for hematologic and biochemical analyses.
- Sample 3: 1 hour after second suckle, for hematologic and biochemical analyses.
- Sample 4: 12 hours from birth, for all the analyses.
- Sample 5: 24 hours from birth, for all the analyses.
- Samples 6 to 11: daily from Days 2 to 7 from birth, for hematologic and biochemical analyses.
- Sample 12: 10 days of age, only for biochemical analysis.
- Sample 13: 14 days of age, only for biochemical analysis.
- Sample 14: 21 days of age, only for biochemical analysis.

For the hematologic analysis (erythrocyte, leukocyte, and platelet counts, together with the related parameters), 2 mL of blood sample was transferred in an EDTA tube, gently mixed, and analyzed within 60 minutes by an automatic cell counter (ADVIA 120; Diamonds Diagnostics, Holliston, MA, USA). The following parameters were analyzed for the erythrocytes count: red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Hct), MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and RBC distribution width (RDW). For the leukocytes count, the parameters analyzed were white blood cell (WBC) count, band and segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Finally, for the platelets count the following parameters were analyzed: platelets count (PLT), medium platelet volume, platelet distribution width, and plateletcrit.

For the clinical biochemistry, a 4-mL aliquot of venous blood was transferred in no-additive tubes, centrifuged at 3000× *g* for 10 minutes, and serum transferred in a plastic tube and analyzed by an automated biochemistry analyzer (Olympus AU 400; Olympus Diagnostica, Hamburg, Germany) within 3 hours after collection.

The following parameters were assessed: alanine aminotransferase (ALT, IU/L), aspartate aminotransferase (AST, IU/L), γGT (IU/L), creatinphospokinase (CPK, IU/L), lactate dehydrogenase (IU/L), alkaline phosphatase (ALP, IU/L), glucose (Glu, mg/dL), blood urea nitrogen (BUN, mg/dL), creatinine (Crea, mg/dL), total serum protein (Tot Prot, g/dL), albumin (Alb, g/dL), cholesterol (Chol, mg/dL), total bilirubin (Tot Bil, mg/dL), calcium (Ca, mg/dL), phosphate (P, mg/dL), magnesium (Mg, mg/dL), sodium (Na, mEq/L), potassium (K, mEq/L), chloride (Cl, mEq/L).