Comp Clin Pathol (2015) 24:335–342
DOI 10.1007/s00580-014-1905-y

ORIGINAL ARTICLE

Hematological variables of the Pêga donkey (*Equus asinus*) breed: influence of age and sex

Annita Morais Girardi · Luiz Carlos Marques · Carmen Zilda Pereira de Toledo · Eugênio de Campos Filho

Received: 21 December 2013 / Accepted: 6 February 2014 / Published online: 22 February 2014

Abstract Appropriate hematological reference intervals mitigate the risk of unnecessary investigations and failures on disease diagnosis. Species, breed, environment, handling, and physiologic stage could influence hematological variables. In this context, there is a lack of hematological reference intervals for Pêga donkeys, an important Brazilian asinine breed. This study aimed to establish hematological reference values for Pêga donkeys and determine the influence of age and sex on these variables. The complete blood count of samples collected from 110 animals, 79 females and 31 males was performed, maintained under field conditions. The animals under 1 year old (three females and five males) were monitored by collections on the day of birth, on 3rd, 7th, 15th day, and monthly until 12 months of life, while the other donkeys were sampled once. There were differences in several variables analyzed, confirming the requirement of different reference intervals according to age group and sex. Moreover, differences among the values obtained and those of previous studies show a breed influence on the hematological variables of donkeys. For Pêga breed donkeys, animal's age and sex influence most of the hematological variables. This study can be useful in the clinical routine and serve as basis to other scientific researches related to Pêga donkeys.

Keywords Asinine · Clinical pathology · Hematology · Pêga · Reference intervals

A. M. Girardi (✉) · L. C. Marques · C. Z. P. de Toledo · E. de Campos Filho
Departamento de Clínica e Cirurgia Veterinária, Faculdade de Ciências Agrárias e Veterinárias, UNESP Univ Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane s/n, CEP 14884-900 Jaboticabal, SP, Brazil
e-mail: annitamgirardi@gmail.com

Introduction

The Pêga donkey (*Equus asinus*), the most popular Brazilian breed, is a big asinine that has Iberian origin, being also popular in some other countries in South America as Bolivia, Paraguay, and Colombia. Its breeding started in Minas Gerais State and, since then, the Pêga breed has been selected for more than two centuries, mainly to produce outstanding saddle-type mules. Nowadays, the Brazilian Association of Pêga Donkey Breeders (ABCJ Pêga) has approximately 2,000 members and about 20,000 mules and donkeys registered (Canisso and McDonnell 2010). However, a limited number of researches about hematological and serum biochemical variables of this species in Brazil have been performed (Campos et al. 1968; Gacek et al. 1973, 1975a, b; Perdigão de Oliveira et al. 1974, 1980, 1983; Godoy 2003; Mori et al. 2003, 2004, 2010a, b; Gravena et al. 2010; Gameleira et al. 2011; Cavalcante et al. 2012; Girardi et al. 2013), and this number is further reduced when speaking specifically of Pêga breed (Campos et al. 1968; Girardi et al. 2013). Much of necessary informations to clinical activities with donkeys are extrapolated from those existing for horses, which could not be a valid comparison (Aluja et al. 2001). Despite belonging to the same family (Equidae) and the same genus (*Equus*), horses and donkeys exhibit very different characteristics in their erythrograms (Perdigão de Oliveira et al. 1974). In general, veterinarians have dispensed little attention to this species, and in most schools of veterinary medicine, donkeys are not included in the study plans (Aluja et al. 2001).

Changes in hematological variables for donkey breeds and populations may be influenced by age, sex, and the time of sampling in relation to exercise, geographical, and nutritional factors (Mori et al. 2004) and should be considered in the clinical analysis of these animals. Often, veterinarians and owners are faced with difficulty in interpreting biological

analysis results from asinines by lack of reference ranges clearly established (Pitel et al. 2006). This study aimed to evaluate the influence of age and sex on hematological reference intervals of Pêga donkeys and obtain data which will guide more accurate interpretation of blood samples from donkeys and serve as basis for future scientific investigations.

Materials and methods

Animals

For this study, blood samples were collected from 110 donkeys, 79 females and 31 males, apparently healthy (based on the animal's history, physical examination, and biochemical analyzes performed concomitantly), maintained under field conditions, bred for reproductive purposes in three donkey herds of São Paulo and Minas Gerais States, Brazil. All farms had the same breeding system, fed on pasture, with supply of feed and mineral mixture when necessary, and appropriated health management.

Sample collection

The animals under 1 year old had blood collected on the day of birth, on 3rd, 7th, 15th day, and monthly until 12 months of life, amounting 124 samples collected from these individuals, with collection period from February 2010 to December 2011. The other donkeys were separated into two age groups: 1 to 3 years (33 animals) and over 3 years old (69 animals), each animal sampled only once, between September 2009 and May 2011.

The collections were performed during the morning, in nonfasting animals, only under mechanical restraint, by closed evacuated system (BD Vacutainer)[1] using multiple sample needles and plastic tubes of 4 mL volume with dipotassium ethylenediaminetetraacetic acid anticoagulant (K_2EDTA 7.2 mg). The collection was conducted by external jugular venipuncture after proper regional antisepsis, up to complete capacity of the tube (Meyer et al. 1995). The samples were mixed after collection and packed in cooler with reusable ice packs for temporary storage and transportation. When they could not be immediately processed, the samples were stored under refrigeration at 4 °C during a maximum period of 12 h (Jain 1993).

Hematological analysis

Red blood cell count (RBC), white blood cell count (WBC), platelet count, packed cell volume (PCV), and hemoglobin concentration (Hb) were measured using an automated veterinary hematology analyzer (ABXVET).[2] The differential leukocyte count (basophils, eosinophils, bands, segmented neutrophils, lymphocytes, and monocytes) was performed by blood smear analysis, stained by modified Rosenfeld method, using light microscopy. The analyses were performed at the Clinical Pathology Laboratory of the Veterinary Hospital "Governador Laudo Natel", School of Agrarian and Veterinary Sciences, São Paulo State University, Jaboticabal, Brazil.

Data analysis

Data analysis was performed using a statistical software (GraphPad InStat version 3.10, 32 bit for Windows).[3] Each parameter was tested for normality applying the Kolmogorov and Smirnov methods. The reference intervals were given as mean ± SD, or median ± SD when the reference intervals did not have a Gaussian distribution, and the 95 % confidence limits. Tukey–Kramer multiple comparison test was used to determine the effect of age on hematological variables for normally distributed data, and if data did not seem to be normally distributed, a nonparametric test (Kruskal–Wallis test and Dunn's multiple comparisons post test) was used. Unpaired *t* tests were used to determine the effect of sex on hematological variables for normally distributed data, and nonparametric tests (Mann–Whitney *U* test) were performed for data not normally distributed. Unpaired *t* tests were also used to compare the present results with hematological reference intervals of donkeys from other studies. Dixon–Reed and Tukey's tests were performed to help the identification of additional outliers using a set of macroinstructions (Reference Value Advisor V 1.4) (Geffre et al. 2011) for spreadsheets (Microsoft Excel).[4] The level of statistical significance was set at $P<0.05$.

Ethical considerations

This study was approved by the Ethics Commission in Use of Animals of the School of Agrarian and Veterinary Sciences, São Paulo State University, Protocol No. 6369/10.

Results and discussion

Reference intervals of hematological variables of Pêga donkey breed determined in the current study are shown in Tables 1 and 2. Comparisons between results of this study and those of previous researches are presented in Tables 3 and 4.

The variables RBC, Hb, PCV, WBC, eosinophils, segmented neutrophils, lymphocytes, monocytes, and platelet counts had Gaussian distribution. Just basophil and neutrophil band

[1] BD Diagnostics—Preanalytical Systems, São Paulo, São Paulo, Brazil
[2] Horiba ABX, Montpellier, Hérault, France
[3] GraphPad Software Inc., San Diego, California, USA
[4] Microsoft, Redmond, Washington, USA

Table 1 Hematological variables of Pêga donkey (*Equus asinus*) breed subtyped by age: under 1 year old (<1), 1 to 3 years old (1–3), and over 3 years old (>3)

Analyte	<1 (*n*=8)	1–3 (*n*=33)	>3 (*n*=69)
RBC ($\times10^{12}$/L)	8.48±1.31 a, 8.25–8.71	7.12±0.93 b, 6.79–7.45	6.02±0.95 c, 5.80–6.25
Hb (g/L)	123.8±18.4 a, 120.6–127.0	120.8±17.3 ab, 114.6–126.9	114.0±15.9 b,110.1–117.7
PCV (L/L)	0.417±0.064 a, 0.406–0.428	0.396±0.053 ab, 0.377–0.414	0.370±0.042 b, 0.359–0.380
WBC ($\times10^{9}$/L)	11.93±3.74 a, 11.26–12.60	10.59±1.98 ab, 9.89–11.30	10.39±2.08 b, 9.89–10.89
Basophils ($\times10^{9}$/L)[a]	0.00±0.06 a, 0.01–0.03	0.00±0.04 a, 0.00–0.03	0.00±0. 04 a, 0.01–0.03
Eosinophils ($\times10^{9}$/L)	0.23±0.17 a, 0.20–0.27	0.32±0.18 a, 0.27–0.40	0.47±0.29 b, 0.41–0.56
Neutrophil band ($\times10^{9}$/L)[a]	0.10±0.15 a, 0.10–0.15	0.00±0.06 b, 0.01–0.05	0.00±0.08 b, 0.04–0.07
Segmented neutrophils ($\times10^{9}$/L)	6.05±2.02 a, 5.69–6.42	5.16±1.41 b, 4.66–5.66	5.07±1.38 b, 4.74–5.40
Lymphocytes ($\times10^{9}$/L)	4.95±2.67 a, 4.48–5.42	4.62±1.28 a, 4.16–5.07	4.43±1.50 a, 4.07–4.79
Monocytes ($\times10^{9}$/L)	0.40±0.20 a, 0.36–0.43	0.35±0.15 a, 0.30–0.41	0.26±0.14 b, 0.23–0.29
Platelets ($\times10^{9}$/L)	345±126 a, 322–367	340±100 a, 305–376	324±114 a, 296–351

Reference intervals are given as mean ± SD (standard deviation) and 95 % confidence limits; analyzed sample size within parentheses; means followed by same letters in rows did not differ significantly at 5 % probability

RBC red blood cells, *Hb* hemoglobin, *PCV* packed cell volume, *WBC* white blood cells

[a] Reference intervals are given as median ± SD (standard deviation) and 95 % confidence limits

counts did not seem to be normally distributed for all age and sex groups.

The RBC decreased with advancing age, as described by several authors (Zinkl et al. 1990; Folch et al. 1997; Caldin et al. 2005; Pitel et al. 2006; Ahmed et al. 2007). The under 1-year-old group had higher Hb than donkeys over 3 years old, as well as reported by researchers for whom this concentration was lower for older animals (Orlandi et al. 1997; Ahmed et al. 2007; Etana et al. 2011). The mean PCV was higher for the under 1-year-old group compared to that for the adult animals, corroborating the descriptions of Pitel et al. (2006), Ahmed et al. (2007), Etana et al. (2011), and Lemma and Moges (2009) that observed lower PCV for older donkeys. These higher erythrocytic values for animals up to 1 year of age were possibly because younger donkeys suffer greater stress during the sampling, resulting in splenic contraction with release of erythrocytes in bloodstream, fact also observed in horses (Pitel et al. 2006).

Animals under 1 year of age show bigger mean of WBC than the group above 3 years old, similarly to reports of other researchers (Dinev and Khubenov 1986; Folch et al. 1997; Caldin et al. 2005; Pitel et al. 2006; Etana et al. 2011) and

Table 2 Hematological variables of Pêga donkey (*Equus asinus*) breed subtyped by sex

Analyte	Female (*n*=79)	Male (*n*=31)
RBC ($\times10^{12}$/L)	6.37±1.08 a, 6.16–6.58	8.22±1.20 b, 7.98–8.45
Hb (g/L)	116.4±16.9 a, 113.3–119.4	124.9±18.1 b, 121.4–128.5
PCV (L/L)	0.382±0.052 a, 0.372–0.391	0.419±0.061 b, 0.408–0.431
WBC ($\times10^{9}$/L)	11.15±2.21 a, 10.74–11.56	10.83±3.19 a, 10.20–11.46
Basophils ($\times10^{9}$/L)[a]	0.02±0.06 a, 0.01–0.03	0.01±0.05 a, 0.00–0.02
Eosinophils ($\times10^{9}$/L)	0.42±0.30 a, 0.37–0.48	0.25±0.18 b, 0.21–0.28
Neutrophil band ($\times10^{9}$/L)[a]	0.08±0.11 a, 0.06–0.10	0.11±0.14 a, 0.08–0.13
Segmented neutrophils ($\times10^{9}$/L)	5.58±1.50 a, 5.31–5.86	5.15±1.58 b, 4.83–5.48
Lymphocytes ($\times10^{9}$/L)	4.70±1.70 a, 4.39–5.02	4.70±2.31 a, 4.25–.15
Monocytes ($\times10^{9}$/L)	0.31±0.14 a, 0.28–0.33	0.36±0.18 b, 0.32–0.40
Platelets ($\times10^{9}$/L)	342±90.6 a, 326–359	333±133 a, 307–359

Reference intervals are given as mean ± SD (standard deviation) and the 95 % confidence limits; analyzed sample size between parentheses; means followed by same letters in rows did not differ significantly at 5 % probability

RBC red blood cells, *Hb* hemoglobin, *PCV* packed cell volume, *WBC* white blood cells

[a] Reference intervals are given as median ± SD (standard deviation) and 95 % confidence limits

Table 3 Comparison of hematological intervals of the Pêga donkey (*Equus asinus*) with other donkey breeds, subtyped by age

<1 year			
	Girardi et al.	Caldin et al. (2005)	Mori et al. (2010a)
RBC ($\times10^{12}$/L)	8.48±1.31	6.55±1.10**	7.68±0.66
Hb (g/L)	123.8±18.4	116.0±16.2	126.4±8.8
PCV (L/L)	0.417±0.064	0.287±0.043**	0.371±0.023
WBC ($\times10^{9}$/L)	11.93±3.74	12.36±3.16	13.99±3.37
Eosinophils ($\times10^{9}$/L)	0.23±0.17	0.54±0.32**	0.57±0.33
Segmented neutrophils ($\times10^{9}$/L)	6.05±2.02	4.5±0.74**	5.57±1
Lymphocytes ($\times10^{9}$/L)	4.95±2.67	6.84±2.5*	7.52±2.5*
Monocytes ($\times10^{9}$/L)	0.4±0.2	0.47±0.4	0.56±0.2
Platelets ($\times10^{9}$/L)	345±126	194±48.6**	
1–3 years			
	Girardi et al.	Perdigão de Oliveira et al. (1974)	Caldin et al. (2005)
RBC ($\times10^{12}$/L)	7.12±0.93	6.62±1.02*	6.27±0.75*
Hb (g/L)	120.8±17.3	122.4±15.4	125.0±13.3
PCV (L/L)	0.396±0.053	0.338±0.046**	0.306±0.035**
WBC ($\times10^{9}$/L)	10.59±1.98		11.52±2.32
Eosinophils ($\times10^{9}$/L)	0.32±0.18		0.5±0.22*
Segmented neutrophils ($\times10^{9}$/L)	5.16±1.41		5.22±1.16
Lymphocytes ($\times10^{9}$/L)	4.62±1.28		5.4±1.35
Monocytes ($\times10^{9}$/L)	0.35±0.15		0.4±0.34
<3 years			
	Girardi et al.	Folch et al. (1997)	
RBC ($\times10^{12}$/L)	8.19±1.36	7.14±1.34**	
Hb (g/L)	123.1±18.2	118.4±14.1*	
PCV (L/L)	0.413±0.062	0.340±0.030**	
WBC ($\times10^{9}$/L)	11.65±3.47	13.9±3**	
Basophils ($\times10^{9}$/L)	0.02±0.06	0.02±0.05	
Eosinophils ($\times10^{9}$/L)	0.37±0.48	0.81±0.71**	
Neutrophil band ($\times10^{9}$/L)	0.11±0.14	0.09±0.14	
Segmented neutrophils ($\times10^{9}$/L)	5.86±1.94	5±1.3**	
Lymphocytes ($\times10^{9}$/L)	4.88±2.45	8±2.7**	
Monocytes ($\times10^{9}$/L)	0.39±0.19	0.27±0.26**	
Platelets ($\times10^{9}$/L)	344±121	229±86.5**	
>3 years			
	Girardi et al.	Folch et al. (1997)	Caldin et al. (2005)
RBC ($\times10^{12}$/L)	6.02±0.95	6.77±1.17**	5.25±0.67**
Hb (g/L)	114.0±15.9	124.0±25.1**	114.0±10.5
PCV (L/L)	0.370±0.042	0.360±0.050	0.285±0.030**
WBC ($\times10^{9}$/L)	10.39±2.08	9.6±1.8**	9.83±2.29
Basophils ($\times10^{9}$/L)	0.00±0.04	0.02±0.06**	
Eosinophils ($\times10^{9}$/L)	0.47±0.29	0.63±0.46**	0.74±0.37**
Neutrophil band ($\times10^{9}$/L)	0.00±0.08	0.08±0.1	
Segmented neutrophils ($\times10^{9}$/L)	5.07±1.38	4.3±1.2**	4.58±1.65
Lymphocytes ($\times10^{9}$/L)	4.43±1.5	4.2±1.2	4.01±1.15
Monocytes ($\times10^{9}$/L)	0.26±0.14	0.21±0.16*	0.32±0.2
Platelets ($\times10^{9}$/L)	324±114	236±82.1**	220±72.1**

Reference intervals are given as mean or median ± SD (standard deviation)

RBC red blood cells, *Hb* hemoglobin, *PCV* packed cell volume, *WBC* white blood cells

Means followed by asterisks in rows differ significantly from those obtained by Girardi et al. (*P<0.05, **P<0.01)

presented the biggest mean of band count, while other age groups did not differ, agreeing with Folch et al. (1997) who observed decreasing in this count with advancing age.

Animals up to 3 years old had lower eosinophil count than adult animals, similar to the reports of Zinkl et al. (1990) and Etana et al. (2011). This increase is probably due to

Table 4 Comparison of hematological intervals of the Pêga donkey (*Equus asinus*) with other donkey breeds, subtyped by sex

	Girardi et al.	Campos et al. (1968)	Mori et al. (2004)	Nayeri (1978)	Orlandi et al. (1997)	Mot et al. (2010)	Etana et al. (2011)
Female							
RBC ($\times 10^{12}$/L)	6.37±1.08	6.27±0.9	6.73±0.67*	8.295±1.511**	5.28±1.44**	6.42±0.133	5.5±1.05**
Hb (g/L)	116.4±16.9	144.5±12.2**	128.0±10.1**	119.2±13.6	136.0±3.3**	117.2±0.9	106.0±36.0
PCV (L/L)	0.381±0.051	0.367±0.024*	0.376±0.029	0.337±0.036**		0.314±0.006**	0.306±0.048**
WBC ($\times 10^9$/L)	11.15±2.21	12.85±2.79**	8.06±1.50**	13.38±3.09**	13.02±3.22**	4.52±0.01**	13.83±3.40**
Bas ($\times 10^9$/L)	0.02±0.06		0.03±0.04**	0.01±0.03		0.03±0.01**	
Eos ($\times 10^9$/L)	0.42±0.3		0.39±0.20	1.23±0.44**	0.17±0.02**	0.84±0.03**	0.72±0.55**
NB ($\times 10^9$/L)	0.08±0.11		0.13±0.14**				
SN ($\times 10^9$/L)	5.58±1.5		3.26±0.77**	5.18±0.92*	4.37±0.08**	1.7±0.03**	5.95±1.25
Lym ($\times 10^9$/L)	4.7±1.7		4.12±0.85**	6.87±1.09**	3.58±0.09**	1.9±0.02**	6.91±1.36**
Mon ($\times 10^9$/L)	0.31±0.14		0.13±0.11**	0.05±0.09**	0.16±0.02**	0.04±0.01**	0.25±0.32
Plat ($\times 10^9$/L)	342±90.6						287±92.5**
Male							
RBC ($\times 10^{12}$/L)	8.22±1.2	6.98±0.7**	7.29±0.50	7.833±1.711	5.77±1.75**	6.38±0.019**	5.7±0.92**
Hb (g/L)	124.9±18.1	157.1±16.2**	132.2±7.3	131.9±19.2*	140.0 ±6.1**	114.5±1.14**	110.0±28.0**
PCV (L/L)	0.419±0.061	0.434±0.049	0.380±0.018**	0.357±0.049**		0.313±0.006**	0.321±0.043**
WBC ($\times 10^9$/L)	10.83±3.19	13.13±2.15*	9.10±1.23*		8.24±4.01**	6.37±0.05**	13.02±2.7**
Bas ($\times 10^9$/L)	0.01±0.05		0.03±0.04	0.0±0.01		0.04±0.01**	
Eos ($\times 10^9$/L)	0.25±0.18		0.32±0.22	1.07±0.54**	0.15±0.03**	0.94±0.07**	0.65±0.38**
NB ($\times 10^9$/L)	0.11±0.14		0.27±0.34				
SN ($\times 10^9$/L)	5.15±1.58		4.09±0.70	5.17±1.08	4.12±0.11**	2.62±0.06**	6.11±1.22**
Lym ($\times 10^9$/L)	4.7±2.31		4.27±0.90	7.41±1.11**	3.82±0.11**	2.73±0.05**	5.99±1.3**
Mon ($\times 10^9$/L)	0.36±0.18		0.13±0.11**	0.08±0.12**	0.16±0.02**	0.05±0.01**	0.20±0.12**
Plat ($\times 10^9$/L)	333±133						309±90.1

Reference intervals are given as mean or median ± SD (standard deviation)

RBC red blood cells, *Hb* hemoglobin, *PCV* packed cell volume, *WBC* white blood cells, *Bas* basophils, *Eos* eosinophils, *NB* neutrophil band, *SN* segmented neutrophils, *Lym* lymphocytes, *Mon* monocytes, *Plat* platelets

Means followed by asterisks in rows differ significantly from those obtained by Girardi et al. (*P<0.05, **P<0.01)

progressive contact of animals with parasite or allergen antigens since their birth, which induct eosinophil poietic factors formation, mainly interleucin 5 (Weiss and Wardrop 2010).

The over 3 years old group showed the lowest mean monocyte count, while the other age groups did not differ, as reported by studies in which observed greater monocyte count for younger animals (Folch et al. 1997; Caldin et al. 2005; Pitel et al. 2006).

Animals under 1 year old showed the highest mean of segmented neutrophil, corroborating with Folch et al. (1997), who reported reduction of this parameter with aging.

As reported by other authors, mean counts of lymphocyte (Gul et al. 2007), platelet (Folch et al. 1997), and basophil (Folch et al. 1997; Gul et al. 2007) did not show age-related differences.

Specifically for animals under 1 year old, the hematological reference intervals were similar to those defined for donkeys in Brazil (Mori et al. 2010a), except the lymphocyte count, that was lower than the limits established by these authors.

The reference limits of Hb for animals between 1 and 3 years old were similar to that described for Brazilian and Italian donkey breeds with 1 to 2 years old (Perdigão de Oliveira et al. 1974), but the values of RBC and PCV were higher in the present study.

The interval of band count was resembling, while the values of segmented neutrophil, monocyte, and platelet counts were higher, and the eosinophil count was lower compared to the intervals established by Folch et al. (1997) for all age groups. For adult animals, the intervals of PCV and lymphocytes were similar, WBC was greater, and RBC, Hb, and basophil count were less than those described by Folch et al. (1997). The interval of basophil count was similar, while for RBC, Hb, and PCV were higher and for WBC and lymphocyte count were lower than the ranges of these authors, for animals up to 3 years old.

The intervals of WBC, Hb, and monocyte were resembling, while RBC, PCV, and platelet count were higher, and eosinophil count was lower than those intervals defined by Caldin et al. (2005), for all age groups. The intervals for lymphocyte and segmented neutrophil counts were similar to those described by these latter researchers for animals older than 1 year old and less than the intervals of these authors for animals under 1 year old.

Although no sex-related differences for hematological variables of donkeys have been reported by Dinev and Khubenov (1986) and Cubeddu et al. (1991), this study showed some discrepant values between sexes.

As reported by other authors, males showed higher means of RBC (Campos et al. 1968; Pitel et al. 2006; Etana et al. 2011), PCV (Nayeri 1978), and Hb (Campos et al. 1968; Nayeri 1978) because female estrogens decrease the erythropoietin production, hormone responsible for the regulation of the erythropoiesis rate (Kerr 2002).

As well as previous studies about the leukogram of donkeys, eosinophil (Campos et al. 1968; Nayeri 1978) and segmented neutrophil (Zinkl et al. 1990) counts were higher for females, mean monocyte count was higher for males (Campos et al. 1968; Nayeri 1978), and several leukocyte counts did not have sex-related differences, such as WBC and lymphocyte (Folch et al. 1997; Al-Busadah and Homeida 2005; Gul et al. 2007; Etana et al. 2011), basophil (Al-Busadah and Homeida 2005; Gul et al. 2007), band (Folch et al. 1997), and platelets (Folch et al. 1997; Etana et al. 2011).

The reference intervals of WBC and Hb, for both sexes, were lower than those described by Campos et al. (1968). For females, the interval of RBC was similar and PCV was higher, while for males, the opposite happened, with resembling PCV and higher RBC intervals than those established by these authors.

The present limits of eosinophil count were similar, but WBC and monocyte counts were greater, for both sexes, than those described by Mori et al. (2004) for Brazilian donkeys breed. For females, the intervals of segmented neutrophil and lymphocyte counts were higher, while RBC, Hb, basophil, and band counts were lower than those described by Mori et al. (2004). The intervals of RBC, Hb, band, segmented neutrophil, and lymphocyte counts were similar, but PCV was greater than the ranges of those described by these authors, for males.

For both sexes, basophil count was resembling, monocyte count and PCV were higher, eosinophil and lymphocyte counts were lower than the intervals of those described by Nayeri (1978). The values of Hb, for females, RBC and segmented neutrophil count, for males, were similar, while the RBC and WBC of females and Hb of males were less than the intervals of those described by this author.

The intervals of RBC, eosinophil, segmented neutrophil, lymphocyte and monocyte counts were higher, while Hb was lower than the intervals of those described by Orlandi et al. (1997), for both sexes. The WBC interval was less, for females, and greater, for males, than those described by these last researchers.

For both sexes, the intervals of WBC, PCV, segmented neutrophil, lymphocyte, and monocyte counts were higher, while basophil and eosinophil counts were lower than those established by Mot et al. (2010). The values of RBC and Hb were similar for females, and greater for males, compared with those of the reports of these authors.

The reference intervals of RBC and PCV were higher, but those of WBC, eosinophil, and lymphocyte counts were lower than those defined by Etana et al. (2011), for both sexes. For females, Hb, segmented neutrophil, and monocyte count intervals were resembling, while the platelet count was greater compared to Etana et al. (2011). The interval of platelet count was similar, Hb and monocyte counts were higher, but

segmented neutrophil was lower than those of these researchers, for males.

Differences were observed in comparisons, by gender, of hematological ranges for Pêga donkeys with other donkey breeds (Nayeri 1978; Orlandi et al. 1997; Mori et al. 2004; Pitel et al. 2006; Mot et al. 2010; Etana et al. 2011) and also within the race (Campos et al. 1968), showing that there are some influential factors on donkey hematological variables such as environment, handling system, feeding, breed, and analysis methodology. It was noticed that about the variables subtyped by age, animals less than 1 year old presented the most discrepant results, reinforcing the importance of specific hematological reference intervals for this age group.

Conclusions

The results obtained indicate that for Pêga breed donkeys, age group and sex of the animal influence most of the hematological variables, and this fact becomes necessary in the establishment of specific reference intervals. Differences among the present results and those of previous researches indicate influences of breed, environment, handling, and analysis methodology on hematological variables of donkeys. The intervals defined in this study are useful for clinical routine and serve as basis for future scientific investigations about these asinines.

Acknowledgments Thanks to the owners of the animals used in this research, Mr. Octávio Junqueira Leite de Moraes and Mr. Gustavo Figueiredo e Mr. José Roberto Silva. This work was performed with scholarship granted by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Process No. 2010/02916-4.

Conflict of interest The authors have declared no conflict of interests.

References

Ahmed AE, Abdel-Hamid HY, Abdel-Rahim AA, Ismail MN (2007) The influence of age and sex on some blood parameters in healthy donkey in south Valley Egypt. Beni-Suef Vet Med J 5:151–158

Al-Busadah KA, Homeida AM (2005) Some physical variables, biochemical and haematological parameters in Hassawi ass. Sci J King Faisal Univ 6(1):145–162

Aluja AS, Bouda J, López AC, Chavira HH (2001) Valores bioquímicos en sangre de burros antes y después del trabajo. Vet Mex 32(4):271–278

Caldin M, Furlanello T, Solano-Gallego L, Lorenzi D, Carli E, Tasca S, Lubas G (2005) Reference ranges for haematology, biochemical profile and electrophoresis in a single herd of Ragusana donkeys from Sicily (Italy). Comp Clin Pathol 14(1):5–12. doi:10.1007/s00580-005-0544-8

Campos JM, Viana ES, Ferreira Neto JM (1968) Hemograma do jumento Pêga. Arq Esc Sup Vet EMG 20:55–58

Canisso IF, McDonnell SM (2010) Donkey breeding behavior with an emphasis on the Pêga breed. In: Matthews NS, Taylor TS (eds) Veterinary Care of Donkeys. International Veterinary Information Service, Ithaca, p No. A2926.0310

Cavalcante PH, Silva ACC, Sakamoto SM, Soto Blanco B (2012) Serum protein fractions in Brazilian breed donkeys using agarose gel electrophoresis. Turk J Vet Anim Sci 36(1):9–12. doi:10.3906/vet-1011-565

Cubeddu GM, Bini PP, Floris B, Carcangiu V, Bomboi G, Pintori G (1991) Hematologic parameters of the white donkeys of Asinara. Boll Soc Ital Biol Sper 67(6):577–584

Dinev D, Khubenov KD (1986) Normal values of the hematological, biochemical and enzymological indices of the donkey. Vet Med Nauki 23(10):69–75

Etana KM, Jenbere TS, Bojia E, Negussie H (2011) Determination of reference hematological and serum-biochemical values for working donkeys of Ethiopia. Vet Res 4(3):90–94. doi:10.3923/vr.2011.90.94

Folch P, Jordana J, Cuenca R (1997) Reference ranges and the influence of age and sex on haematological values of the endangered Catalonian donkey. Vet J 154(2):163–168

Gacek F, Bizutti O, Perdigão de Oliveira FRA, Leão JFS (1973) Eletroferograma das proteínas séricas de jumentos Equus asinus L. normais da raça Brasileira. Bol Ind Anim 30(1):161–171

Gacek F, Bizutti O, Perdigão de Oliveira FRA, Leão JFS (1975a) Analyse électrophorétique des protéines sériques d'ânes (Equus asinus L.) normaux de la race Italienne. Arch Vet Ital 26(5–6):189–197

Gacek F, Ferri RG, Bizutti O (1975b) Gamma G(T)-like globuln in ass (Equus asinus L.) serum and its behaviour during pregnancy. Comp Biochem Physiol 50A:565–567

Gameleira J, Sousa RS, Barros IO, Tavares MD, Firmino PR, Souza FJA, Barrêto Júnior RA (2011) Parâmetros hematológicos de asininos (Equus asinus) da raça nordestino. In: 38° Conbravet, Florianópolis, November 1–4 2011

Geffre A, Concordet D, Braun JP, Trumel C (2011) Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Pathol 40(1):107–112. doi:10.1111/j.1939-165X.2011.00287.x

Girardi AM, Marques LC, Toledo CZP, Barbosa JC, Maldonado Jr. W, Jorge RLN, Nogueira CAS (2013) Biochemical profile of the Pêga donkey (Equus asinus) breed: influence of age and sex. Comp Clin Pathol:1–7. doi:10.1007/s00580-013-1718-4

Godoy RF (2003) Estudo eritroleucométrico e proteinograma do sangue do cordão umbilical e da jugular de equinos e asininos ao nascimento e de suas respectivas mães Dissertation, Universidade Estadual Paulista, Jaboticabal

Gravena K, Sampaio RCL, Martins CB, Dias DPM, Orozco CAG, Oliveira JV, Lacerda-Neto JC (2010) Parâmetros hematológicos de jumentas gestantes em diferentes períodos. Arq Bras Med Vet Zootec 62(6):1514–1516

Gul ST, Ahmad M, Khan A, Hussain I (2007) Haemato-biochemical observations in apparently healthy equine species. Pak Vet J 27(4):155–158

Jain NC (1993) Essentials of veterinary hematology. Lea & Febiger, Philadelphia

Kerr MG (2002) Veterinary laboratory medicine - clinical biochemistry and haematology, vol 2. Blackwell Science, Oxford. doi:10.1002/9780470690246

Lemma A, Moges M (2009) Clinical, hematological and serum biochemical reference values of working donkeys (Equus asinus) owned by transport operators in Addis Ababa, Ethiopia. Livest Res Rural Dev 21(8):#127

Meyer DJ, Coles EH, Rich LJ (1995) Medicina de laboratório veterinária: interpretação e diagnóstico. Roca, São Paulo

Mori CK, Melo GD, Almeida BFM, Bomfim CAM, Meira C, Kohaygawa A (2010) Bomfim SRM Avaliação hematológica de jumentos (Equus asinus) do nascimento a um ano de idade. In: 1° Simpósio de Pós-Graduação em Ciência Animal e IX Semana de

Divulgação Científica do Curso de Medicina Veterinária da Unesp de Araçatuba-SP, Araçatuba, 2010a. Veterinária e Zootecnia, p 122

Mori CK, Melo GD, Ciarlini PC, Perri SHV, Bomfim CAM, Meira C, Kohaygawa A (2010) Bomfim SRM Influência da idade nos níveis de proteína total plasmática em jumentos (Equus asinus). In: 1° Simpósio de Pós-Graduação em Ciência Animal e IX Semana de Divulgação Científica do Curso de Medicina Veterinária da Unesp de Araçatuba-SP, Araçatuba, 2010b. Veterinária e Zootecnia, p 129

Mori E, Fernandes WR, Mirandola RMS, Kubo G, Ferreira RR, Oliveira JV, Gacek F (2003) Reference values on serum biochemical parameters of Brazilian donkey (Equus asinus) breed. J Equine Vet Sci 23(8):358–364

Mori E, Mirandola RMS, Ferreira RR, Oliveira JV, Gacek F, Fernandes WR (2004) Reference values on hematologic parametes of the Brazilian donkey (Equus asinus) breed. J Equine Vet Sci 24(7): 271–276. doi:10.1016/j.jevs.2004.06.008

Mot T, Mot D, Petruse C, Morar D, Ciulan V, Simiz F, Ciorba G, Panait M (2010) Hematological investigations in donkeys. Lucrari Stiintifice Med Vet 43(1):331–333

Nayeri GD (1978) Blood characteristics of the adult donkey. Zentralbl Vet Med A 25(7):541–547

Orlandi M, Leotta R, Berni P, Curadi MC (1997) Profilo metabolico nell'asino amiatino [Toscana]. Ann Fac Med Vet Pisa 50:47–53

Perdigão de Oliveira FRA, Augusto C, Grasso PL, Souza H, Baudet GJA (1974) Eritrograma normal de jumentos Equus asinus das raças Puro-sangue Italiana e Brasileira de 1 a 2 anos de idade. Bol Ind Anim 31(2):325–329

Perdigão de Oliveira FRA, Gacek F, Cury L, Augusto C, Leão JFS (1980) Evolução do perfil soro-protéico em asininos e muares, durante o desenvolvimento. Bol Ind Anim 37(2):245–256

Perdigão de Oliveira FRA, Gacek F, Leão JFS, Vieira JM, Augusto C (1983) Profile of globulins in the serum of pregnant donkeys. Bol Ind Anim 40(2):219–227

Pitel P, Moulin M, Valette JP, Dumontier S, Petit L, Fortier G, Coroucé-Malblanc A (2006) Approche des valeurs hématologiques et biochimiques chez deux races asines. Prat Vet Equine 38(149):19–25

Weiss DJ, Wardrop KJ (2010) Schalm's Veterinary Hematology, 6th edn. Wiley-Blackwell, Ames

Zinkl JG, Mae D, Guzman Merida P, Farver TB, Humble JA (1990) Reference ranges and the influence of age and sex on hematologic and serum biochemical values in donkeys (Equus asinus). Am J Vet Res 51(3):408–413