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Original

Hematological parameters in peruvian paso horses in the Lambayeque province

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ABSTRACT

Objective. To determine the haematological parameters according to the reproductive status of Peruvian Paso horses in the Province of Lambayeque. **Materials and methods**. The research was conducted on 60 clinically healthy adult horses (> 4 years), without previous drug treatment. Four groups of 15 horses were formed: gelding, entire male, empty mare and pregnant mare. First, a general clinical examination was performed to confirm their good health. Blood was drawn from the jugular vein of all the horses. The samples were processed by haematological analysis and the parameters of the red series (haemoglobin, haematocrit, red blood cells, mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH] and the mean corpuscular haemoglobin concentration [MCHC] were determined. Likewise, the white series (leukocytes, neutrophils segmented and stacked, basophils, monocytes, lymphocytes, eosinophils) and the thrombocytic series were processed and the unit range determined. Manual methods, mathematical formulas and the Drabkin method were used in the clinical pathology laboratory. The Kruskal Wallis Statistical test was applied and consequently the mean, standard deviation and ranges of variables were obtained. **Results.** No significant statistical differences were found in the white, red and thrombocytic series according to sex or reproductive status, except the number of basophils was higher in the entire males. **Conclusions.** The haematological parameters according to the reproductive status of the Peruvian Step horse are within the normal ranges (as per the literature pertaining to this area). However, the number of lymphocytes was much lower.

Keywords: Horses; reproductive status; haematological parameters (Source: *DECs*)

RESUMEN

Objetivo. Determinar los parámetros hematológicos según el estado reproductivo en caballos peruanos de Paso en la provincia de Lambayeque. **Materiales y métodos**. Se realizó la investigación con 60 caballos adultos (>4 años), clínicamente sanos y sin tratamiento farmacológico previo; según su estado reproductivo se formaron cuatro grupos de 15 ejemplares cada uno: macho castrado, macho entero, yegua vacía y yegua gestante. Primero se realizó el examen clínico general para confirmar su buen estado de salud, luego se extrajo sangre de la vena yugular a todos los caballos.

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Las muestras se procesaron por análisis hematológicos y se determinaron las variables de la serie roja (hemoglobina, hematocrito, glóbulos rojos, volumen corpuscular medio [VCM], hemoglobina corpuscular media [HCM] y concentración de hemoglobina corpuscular media [CHCM]). Igualmente, la serie blanca (leucocitos, neutrófilos segmentados y abastonados, basófilos, monocitos, linfocitos, eosinófilos) y serie trombocítica fueron procesados y determinados. Se utilizaron métodos manuales, fórmulas matemáticas y método Drabkin en el laboratorio de patología clínica. Se aplicó la prueba estadística de Kruskal Wallis y se obtuvo la media, desviación estándar y rangos de las variables. **Resultados**. No se encontraron diferencias estadísticamente significativas en la serie blanca, roja y trombocítica de acuerdo con el sexo o estado reproductivo, a excepción del número de los basófilos que fue mayor en machos enteros. **Conclusiones.** Los parámetros hematológicos según el estado reproductivo del caballo peruano de paso se encuentran dentro de los rangos establecidos por la literatura especializada. Sin embargo, el número los linfocitos que fue mucho menor.

Palabras clave: Caballos; estado reproductivo; parámetros hematológicos (*Fuente: DECs*).

INTRODUCTION

Since time immemorial, the horse has been used by mankind to carry out many tasks and in recent decades horses have proved helpful to counteract psychomotor disabilities and can act as a mitigating factor in children with cerebral palsy (1,2).

The haematological, enzymatic, or biochemical parameters can be highly variable in horses around the world; depending on age, race, sex, type of work, temperature or some pathological condition. In the case of high-performance endurance horses, potassium and lactate levels are higher compared to those (horses) of lower performance (3) likewise, haemoglobin, iron and copper values are lower in those horses that are sodium deficient (4). In adult equines over 15 years, concentrations of plasma proteins, leukocytes, neutrophils, haemoglobin, and haematocrit are higher compared to those of a younger age and exposed to constant exercise (5, 6). After exercise, some females may present changes in their haematological profile. (7). Similarly, the environment might influence serum biochemical parameters, especially in analytes such as AST (Aspartate aminotransferase) and CK (creatine kinase) in horses bred in the tropics (8).

Haematological values are an important tool to describe the general physical condition of the horses and to determine a possible prognosis in a given pathology (9,10). But to date, there is no evidence of the use of these values as a diagnostic tool for the evaluation of the Peruvian Paso horse, according to its reproductive status, being used as a reference in clinical practice. This has been one of the major barriers that veterinarians dedicated to the equine medical clinic have had to face.

This study aimed to determine the normal haematological parameters of the different reproductive stages of the Peruvian Paso horse owing to the lack of sufficient information in the current literature.

MATERIALS AND METHODS

Study site. This cross-sectional, observational study was conducted in the months of January and February 2019, in different stud farms belonging to the Association of Breeders and Owners of Peruvian Paso horses in the Province of Lambayeque - Perú. The area is located in the north of the country and has an average temperature of 72.5°F, relative humidity of 48% and 18 metres (59.06 feet) above sea level, located between the coordinates 6° 42’17” S 79° 54’25” W.

Selection of animals. Those selected were 60 equines with good body condition, older than 4 years (with an average age between ±3.9 and 8.8 years), with free access to water and with an equal proportion of males and females; differentiated by their reproductive status in four research groups of 15 animals in each group: empty female (G1), pregnant female (G2); gelding (G3) and entire male (G4). The diet mainly consisted of alfalfa (Medicago sativa), sorghum fodder (Sorghum sp.) and maralfalfa (Pennisetum sp.) in a proportion of 2% to their body weight, together with concentrates (Omolin® by Purina).

Handling of animals prior to sampling and criteria for exclusion. Prior to taking blood samples, the animals were fasting and had a 30-minute rest. They underwent a general clinical examination in order to validate their

precise state of health and average age using dental chronometry. Horses with pharmacological treatment during the three months prior to taking blood samples or with the presence of any pathology were not included in the study.

Clinical examination. The clinical examination methods used were based on inspection, palpation, percussion and auscultation. The animals were observed from the front, on both sides and from behind to acquire a better assessment of the overall appearance of the body, posture, temperament, signs of pain, weaknesses, condition of skin and hide. In the same way, the respiration rate and depth, existence of wounds, inflammations, asymmetries in muscle development and possible exudates present in the natural orifices were analysed. Attention was given to the oral and ocular mucous membranes, looking for abnormalities in colour, texture, and dental conformation. The pre-scapular and pectoral regions of the forelimbs were palpated seeking the existence of hot areas, pain or inflammation, as well as conformational abnormalities. Heart rate (HR) and respiratory frequency (RF), digital pulse, and body temperature were calculated. The normal physiological ranges for this species were taken as a reference: HR=28-40 beats/min, RF=8-20 breaths min and body temperature =99.5–101.3°F. Examination of the abdomen was performed by auscultation of the paralumbar fossa and the ventral flank regions to evaluate peristaltic movements and sounds from the ileocecal valve (borborygmi). This procedure allowed the evaluation of intestinal sounds (normal, increased, decreased or absent). In the right ventral region, the right ventral colon was auscultated; in the left paralumbar fossa, the small intestine; and in the left ventral region, the left ventral colon. In the case of mares, the vulva was examined (conformation, tone and colour of the mucous) in search of possible abnormal discharges. In contrast, in the males, the foreskin, penis and testicles were palpated to verify the existence or not of neoplasms (11).

Blood sampling. Before taking the samples, antisepsis was performed in the jugular sulcus area with 90% alcohol. Three mls of blood was taken from each animal, using a Holder and a needle (21x $1^{1/2'}$), in a collection tube containing EDTA anticoagulant (ethylenediaminetetraacetic acid). The samples were homogenized and labelled indicating the age, sex and provenance of the animal. The samples were refrigerated (37.4–41^{0}F = (3-5^{0}C) in an airtight container (Cooler) with cooling gel and transported to the Clinical Pathology Laboratory of the Veterinary Medicine Faculty of the "Universidad Nacional Pedro Ruiz Gallo". The time between blood sampling and laboratory analysis was on average 45 min.

Analysis of blood samples. To determine the haematocrit (Hct), heparinized capillary tubes were used which were sealed at one end with plasticine. They were then centrifuged for three minutes at 30000 rpm in a micro centrifuge. Results were interpreted according to the haematocrit table 0-100 and expressed as a percentage (12).

Haemoglobin (Hb) was calculated through the homogenization of 2.5 ml of Drabkin reagent plus 10 µL of blood that were left to rest for a period of three minutes. The test tubes were read in the photo colorimeter at a wavelength of 550 nm, using a white tube with the Drabkin solution that served as a calibrator (12).

The total erythrocyte (fT/bT) count was carried out through the Neubauer chamber. For this, 1 ml of Gower reagent was diluted with 5 µL of blood with anticoagulant. The tubes were carefully inverted several times in order to achieve a better mixture and allowed to rest for 3 minutes. 10 µL of the solution was introduced between the Neubauer chamber and the coverslip to permit it to flow through the capillary and then allow it rest for another 3 minutes. Counting was performed under a 40X light microscope, using the following formula: cells counted x 10000 (12).

Corpuscular constants were derived using the following formulas:
MCV = Hct x 10/fT; MCH = Hb x 10/fT and
MCHC = Hb x 100 / Hct (12.13).

To count the Leukocyte Total, 0.96 ml of Turk's reagent and 50 µL of blood were mixed and left to repose for 3 minutes. Then, 10 µL of the solution was introduced by capillarity between the Neubauer chamber and coverslips and allowed to rest for another three minutes for a later count. The following formula was followed: Leukocyte Total = counted cells x 50 (12).

The differential leukocyte count was performed on blood smears using Wright's stain. A total of 100 cells were counted and the quantities observed were expressed as a percentage. The myeloid and lymphoid precursors were grouped to facilitate the separation in the leukocyte

formula and to establish the comparison with the reference physiological ranges (12,13).

To determine the total platelets count (TPC), 2 ml of ammonium oxalate and 20 µL of blood were mixed. Subsequently, 10 µL of the solution was placed in the Neubauer chamber, which was left to incubate in a humid chamber for a period of 15 minutes. TP = cells counted X 1000 (12).

Statistical analysis. The Levene and Kruskal Wallis tests of homogeneity of variance were applied. The Tukey's post hoc test was applied to evaluate possible differences between study groups (reproductive status and sex).

Each group was considered statistically different when the value (was) $p<0.05$. The statistics of each variable of the red, white and thrombocytic series were obtained as the standard deviation, mean and ranges. The Statistical Package Statistics v25.0, SPSS was used.

Ethical aspects. All procedures carried out, during sample collection and general physical examination were within the limits allowed in the regulations of the Institutional Committee for Ethics in Animal Research and Biodiversity of the "Universidad Científica del Sur", approved in 2017. In this study the integrity and the health of the animals evaluated was safeguarded.

RESULTS

The haematocrit, haemoglobin, and red blood cell count were higher but not statistically significant in entire males as shown in table 1. Furthermore, no significant differences were found in the red blood cells series in relation to the reproductive status of the horses.

In the values of the white series (Table 2), the values of leukocytes and segmented neutrophils were lower in males, but higher in stocked neutrophils. Furthermore, there is statistical significance among the groups favouring the entire males that showed greater number of basophils.

Table 1. Haematological values of the red series among the research groups of equines of the Peruvian Paso horse bred in Lambayeque.

Red series	Groups				Average (max-min)	p Value*
	G1 (n=15)	G2 (n=15)	G3 (n=15)	G4 (n=15)		
RBC (X10^6/µl)	7.3 ± 0.8	7.5 ± 1.1	7.7 ± 0.9	8.0 ± 1.1	7.6 ± 1.1 (5.7 - 9.3)	0.26
Hb (g/dL)	10.8 ± 1.8	11.4 ± 2.3	11.6 ± 1.9	12.4 ± 2.2	11.6 ± 2.1 (8.0 - 15.4)	0.22
Hct (%)	35.3 ± 4.1	36.4 ± 5.0	36.9 ± 3.8	38.7 ± 4.9	36.8 ± 4.5 (28.0-45.0)	0.24
MCH (pg)	14.7 ± 0.7	15.1 ± 1.1	15.0 ± 0.8	15.4 ± 1.0	15.0 ± 0.9 (13.4 -6.6)	0.28
MCV (fl)	48.6 ± 0.6	48.6 ± 1.9	48.0 ± 2.2	48.5 ± 1.8	48.4 ± 1.7 (41.9-53.2)	0.77
MCHC (g/dl)	30.3 ± 1.7	31.1 ± 2.3	31.4 ± 2.2	31.8 ± 2.2	31.2 ± 2.1 (27.7-35.3)	0.27

G1=empty female; G2=pregnant female: G3 =gelding; G4=entire male; Min=Minimum; Max=Maximum; RBCs=Red blood cells; Hb=Haemoglobin; Hct=Haematocrit; MCH=mean corpuscular haemoglobin; MCV=mean corpuscular volume; MCHC=Mean Corpuscular Haemoglobin Concentration.
*$p<0.05$ in relation to reproductive status (Kruskal-Wallis).

Table 2. Haematological values of the white and thrombocytic series among research groups of equines of the Peruvian Paso horse bred in Lambayeque.

White series	Groups				Average (max - min)	p Value*
	G1 (n=15)	G2 (n=15)	G3 (n=15)	G4(n=15)		
Leu (/µL)	10367±1529	10470±2107	9687±2166	9667±1690	10048**±1881** (5700 - 13500)	0.51
Segs (/µL)	5037 ± 1147	5384 ± 1834	4970±1315	4944±1255	5084±1388 (2622 - 8190)	0.81
Ab (/µL)	39 ± 50	35 ± 46	68 ± 45	63 ± 48	51 ± 48 (0 - 122)	0.14
Eos (/µL)	370 ± 337	444 ± 278	224 ± 176	388 ± 293	357 ± 282 (0 - 976)	0.17
Bas (/µL)	19 ± 41	51 ± 73	54 ± 66	82 ± 57 [a]	52 ± 63 (0 - 239)	0.04
Lymp (/µL)	4795 ± 1080	4343 ± 857	4223± 1356	4086±1131	4362±1124 (1650 -6750)	0.35
Mo (/µL)	144 ± 111	212 ± 125	148 ± 140	104 ± 98	152 ± 123 (0 - 515)	0.11
Tc (mil/µL)	167 ± 25	178 ± 21	173 ± 21	168 ± 20	171 ± 22 (120 - 213)	0.46

G1=empty female; G2 = pregnant female; G3 = gelding; G4 = whole male; WBCs = Leukocytes; Segs = Segmented neutrophils; Ab = stockpiled neutrophils; Eos = Eosinophils; Bas = Basophils; Lymp = Lymphocytes; Mo = Monocytes; Tc = Thrombocytes; *p<0.05 in relation to reproductive status (Kruskal-Wallis).
[a] = different from the rest of the group according to Tukey's post hoc test.

DISCUSSION

In veterinary medicine, haematological examinations are the most requested by professionals due to their speed and simplicity. The results give an overview of the state of health of the animal which is complemented by the general physical examination and medical history. Haematological values are highly variable; nutrition, age, race, among other factors may be the causes of these variations. However, the findings found in this study are located within the parameters reported by specialized literature and prestigious Peruvian laboratories that perform clinical analyses on this species. It is reported that the number of lymphocytes are below that reported by the laboratories (13,14).

The total count of red blood cells found is lower than that found in horses from the Lurín Valley with an average of 8.3 x 106 / µL (15). The haematocrit was found in the lower limit of Creole horses in Brazil, with an average of 37.4% (16); but above that found in Friesian horses from North America (34%) and the Japanese Noma race (35.5±7.1%). Furthermore, the latter has high levels of eosinophil (383±529 / µl) and low levels of monocytes (74±123 / µl) (17,18). Possibly the aforementioned contrasts are due to differences in health protocols, exposure to diseases, altitude of the region and diet. The diet probably being the cause of haematological changes in horses (19).

Haemoglobin peaks can differ in the same animal according to the length of exercise time it completes. This explains why the haemoglobin values of the Peruvian Paso horse are very similar to those found in the Jeju breed horses older than 4 years when at rest (20).

The level of basophils in entire males (82±57/µl) was the highest within the groups studied. This value is above those found in 10-year-old males of the Barb breed in Algeria, with an average of

53 ± 47.51 / µl, where it was also shown that the number of basophils was higher in males (21). Although it is common for the equine species to register basophilia (12).

Regardless of the fact that in this study there were no significant differences between the sexes, it was confirmed that females have greater variability in haematological parameters after exercise (7), although in some races (Lipizzano) this may have the opposite effect (22). The same could occur with MCV, where this is sometimes higher in castrated males, older than 25 months (23). However, no differences were observed among the groups studied.

The MCV is very similar to that of healthy Arabian horses; but it is located below the MCH and MCHC levels, with an average of 17.5 pg and 36.64 gr/dl, respectively. Furthermore, platelet levels were much higher compared to the aforementioned breed (24).

Likewise, pregnant mares in peripartum may have different haematological values than the rest of the group due to dehydration in foaling, which causes haemoconcentration, stress and energy imbalance (25,26). In contrast, the opposite can occur in empty mares that have higher values in haemoglobin and red blood cell count, compared to Andalusian and thoroughbred pregnant mares (27). On the other hand, the clinically healthy heavy draft mares from Japan have lower lymphocyte values (2490/µl) but a high number of segmented neutrophils (5730 /µl) compared to the pregnant Peruvian Paso mare (28). In contrast to these findings, in this study no differences were observed in the same variables.

However, the sampling of the present study was carried out in the summer season, a time during which some authors have verified the existence of haematological changes caused by the temperature of the environment, relative humidity and rainfall where the MCH is highest in summer and eosinophils in winter (29). Therefore, the aforementioned factors must be considered to define the extraction of blood samples.

As is known, the Peruvian Paso horse is not used exclusively for work, but rather for exhibitions and competitions. During the time prior to such events, the horses undergo training for long periods. Haematological and biochemical changes can be perceived relative to those horses not subjected to these intense practices and exercises (30,31,32). This could be a conditioning factor for changes in haematological values in the Peruvian Paso horse.

As had been pointed out, there are no haematological parameters in reference to the Peruvian Paso horses based on their reproductive status. The results obtained will serve as a starting point for developing a comprehensive normal range of parameters and in turn, facilitate a prognosis on the general health of horses of Lambayeque.

The conclusion is that the haematological parameters studied in the Peruvian Paso horses of the Lambayeque Province remain within the reference physiological range and are similar in the different reproductive states.

Conflict of interest

The authors declare that they have no conflict of interest.

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