



DETERMINATION OF THE REFERENCE VALUES IN THE BLOOD COUNT OF HORSES BORN OR REARED BETWEEN 0 AND 500 M.A.S.L. IN THE COASTAL REGION OF ECUADOR

DETERMINACIÓN DE LOS VALORES DE REFERENCIA EN EL HEMOGRAMA DE CABALLOS NACIDOS O CRIADOS ENTRE 0 Y 500 M.S.N.M. EN LA REGIÓN LITORAL DEL ECUADOR

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Abstract

The present study was carried out in the littoral region of Ecuador, analyzing the hemograms of 100 horses clinically healthy, over two years of age and reared between 0 and 500 masl, in order to determinate hematological reference values and compare the results obtained with the reference values of a previous study performed to more than 3000 masl, in the Ecuadorian north central sierra. Therefore, blood samples were taken from animals at rest and were submitted to laboratory analysis with the Mindray®auto-hematology equipment BC2800Vet, and with this information the statistical study was made using Microsoft Excel® "Reference Value Advisor" to identify possible references values and to eliminate outlier data. These blood samples were taken from Ecuadorian creole horses in order to maintain a statistical sample with homogeneous blood parameters and a reference of this variety, which has only been studied before over 3000 masl. The following values were identified: erythrocytes: $4.90-9.38 \times 10^6/\mu\text{L}$, hematocrit: 24.83-45.10%, hemoglobin: 8.59-14.87g/dL, mean corpuscular volume: 42.35-55.19fL, mean corpuscular hemoglobin: 14.25-18.20pg, concentration of mean corpuscular hemoglobin: 32.10-36.70g/dL, white blood cells: $5.64-12.81 \times 10^3/\mu\text{L}$, lymphocytes: $1.04-5.85 \times 10^3/\mu\text{L}$, monocytes: $0.20-0.90 \times 10^3/\mu\text{L}$, granulocytes: $2.90-8.26 \times 10^3/\mu\text{L}$, and blood platelets between: $78.10-314.90 \times 10^3/\mu\text{L}$. In the comparison with the obtained results, significant differences were found ($P < 0.05$) in the erythrocytes, hemoglobin, hematocrit and platelets reference values due to the height influence; while significant differences ($P < 0.05$) were also found in the white series (leukocytes, lymphocytes, monocytes and granulocytes) but this due to physiological or pathological influence and not thanks to the altitudinal effect.

Keywords: altitude, hemogram, hypoxia, horses.

Resumen

El presente trabajo se realizó en la región litoral del Ecuador, en donde se analizaron los hemogramas de 100 caballos criollos clínicamente sanos, mayores a dos años de edad y criados entre los 0 y 500 metros sobre el nivel del mar, para cumplir con los objetivos de determinar los valores hematológicos de referencia y comparar los resultados obtenidos con los valores de referencia de un estudio previo, realizado a más de 3000 m.s.n.m en la sierra centro norte ecuatoriana. Se obtuvieron las muestras sanguíneas de animales en reposo, se realizaron los análisis de laboratorio con el equipo de auto-hematología Mindray®BC2800Vet y posteriormente se hizo un análisis estadístico utilizando el complemento de Microsoft Excel® "Reference Value Advisor" para determinar los valores de referencia y eliminar los valores anómalos. Se prefirió tomar la muestra de caballos criollos ecuatorianos para tener una muestra con parámetros sanguíneos homogéneos y una referencia de esta variedad antes solamente estudiada sobre los 3000 m.s.n.m. Se encontraron los siguientes valores; eritrocitos: 4,90-9,38x10⁶/mL, hematocrito: 24,83-45,10%, hemoglobina: 8,59-14,87g/L, VCM: 42,35-55,19fL, HCM: 14,25-18,20pg, CHCM: 32,10-36,70g/L, glóbulos blancos: 5,64-12,81x10³/mL, linfocitos: 1,04-5,85x10³/mL, monocitos: 0,20-0,90x10³/mL, granulocitos: 2,90-8,26x10³/mL y plaquetas: 78,10-314,90x10³/mL. En la comparación con los resultados obtenidos se encontraron diferencias significativas (P<0,05) en el conteo de eritrocitos, concentración de hemoglobina, hematocrito y conteo de plaquetas debido a la influencia de la altura; también se encontraron diferencias significativas (P<0,05) en la serie blanca (leucocitos, linfocitos, monocitos y granulocitos) pero esto debido a influencias fisiológicas o patológicas, mas no al efecto altitudinal.

Palabras claves: altura, caballos, hemograma, hipoxia, mar.

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1 Introduction

Creole horses are animals developed in America with a great physical strength; this breed of horses has been able to adapt since times of the conquest, 500 years ago, to the various lands of America, from the high moorlands to the beaches, even the Amazon and the rugged forests. The study and interest on the creole horse originated many years ago due to the implementation of new equine breeds introduced in the country.

These new and different breeds turned out to be larger and faster. However, with the passing of the years, crosses between these breeds and the creole horses were carried out, which later resulted in the improvement of the interest characteristics to the man along with a better adaptability to the environment (Almeida Sosa, 2012; Bravo Intrigao, 2013).

The Ecuadorian creole horse is described as a small animal, between 1.35 and 1.45 meters, sturdy, with great resistance, ideal for moorlands because it supports great physical efforts and variations in height (Bravo Intrigao, 2013).

The medicine at different heights has great importance in veterinary medicine as well as in the human medicine, since it is important to know how the variation of the atmospheric pressure influences at different altitudes on the sea level, and on the organism of the animals, in this case equines (Suarez, 2001).

Several aspects of the environment vary as altitude increases or decreases, such as barometric pressure (PB) or also known as atmospheric pressure (PA), oxygen partial pressure (PO_2), temperature (T°), feeding, among others (Cárdenas, 20003; Usamayta Quispe, 2007).

In the country, a previous study related to this topic is the Izurieta Barzola *et al.* (2017), so it is considered of utmost importance to complement it with a study done at the sea level, under the same parameters and taking into account that the cardiovascular system suffers physiological compensatory adjustments at different heights and in the case of equines a decompensation is reflected in the appearance of several physiological alterations (Quintela, 1985; Izurieta Barzola *et al.*, 2017).

The blood count is one of the basic tests most used in the equine medical clinic, as it is an indicator of alterations that cannot always be perceived in conducting the clinical examination; therefore, the evaluation of the cellular elements of the

blood, both quantitatively and qualitatively, could serve as a diagnostic method for a specific disease, but most of the time it is used to know the general health condition of an individual and provide essential information about the control of diseases (Cuenca Valera y Pastor Milán, 2006; Kazuko *et al.*, 2009). The blood count (Mejía, 2014) is also included for the pre-anesthetic horse profile.

The results of a blood count should always be interpreted taking into account the general state of the patient (age, breed, sex, aptitude, previous medical treatments, environment in which the horse inhabits, physical clinical examination, management, state of excitement, form of sample collection, applied methodology analysis, etc.), since any alteration in any of these variants can significantly modify the results and interpretation of the CBC (Arias Gutiérrez y Pérez Jaramillo, 2006; Cuenca Valera y Pastor Milán, 2006).

The CBC comprises the quantification of blood components, including a erythrocyte count, hematocrit value, hemoglobin concentration, medium corpuscular volume (VCM), corpuscular mean hemoglobin concentration (MCHC), total and differential count of leukocytes and platelet count (Arias Gutiérrez y Pérez Jaramillo, 2006).

The objectives of this work were to determine the values of normal hematological parameters of horses born or reared between 0 and 500 masl in the coastal region of Ecuador and compared them with the reference values of a previous study, conducted at more than 3 000 masl in the north central Ecuadorian moorlands and in the same breed of horses.

There are hematological parameters established for the equine species, however, these have not considered the breed or the different variations in height in which these animals are; in addition, the values reported have been established in other countries, reason for which it is necessary to determine the reference parameters in the blood count of Ecuadorian creole horses at sea level in the coastal region of Ecuador to have a basis in the physiological control of these animals; this will allow to find differences in the reference blood values by the effect of altitude variation, which will facilitate a better appreciation of the health for future patients depending on the area.

2 Materials and methods

The study was carried out in the coastal region of Ecuador, in eight zones between 0.88 and 466 masl where were taken 100 samples that are detailed below: Santo Domingo de los Tsachilas: Santo Domingo 9. Esmeraldas: Sua 22, Quinde 13, La Unión 16. Los Ríos: Quevedo 10. Manabí: Tosagua 14, Briceño 6 and San Isidro 10. Most of the equines sampled belonged to small communities, where the animals were used as means of work and transportation; small farms were also part of the sampling where the animals had the same function.

2.1 Factors under study

In the present study the factor was the height between 0 and 500 masl, as it was sought to determine whether the normal values in the blood of Creole horses born or reared at this altitude vary compared to Creole horses born or reared to more than 3 000 masl. The study consisted of taking external jugular venous blood samples from Creole horses (males and females) clinically healthy and over 2 years, and then were processed in the laboratory.

Blood counts were made in all the samples using the Mindray®BC-2800Vet Auto-hematology equipment to determine the reference values of each hematologic parameter. In order to fulfill the second specific objective of this research, the results of the reference values in the horse's blood count were compared between 0 and 500 masl obtained in this study, compared to the reference values in the blood count to more than 3 000 masl from an earlier study carried out in Ecuador; this through a statistical analysis.

The sample of animals in this investigation correspond to the horses used to take blood samples, which were selected under the following inclusion criteria: horses born or reared at an altitude between 0 and 500 masl in the coastal region of Ecuador, these were between males and females older than 2 years of old and in good health; these were animals used for work and were preferred Ecuadorian creole horses to avoid variations in their blood composition parameters by effect of the breed.

2.2 Statistical analysis

To carry out the statistical analysis of this study, a program was used that allowed to determine the

reference limits or reference intervals (IR) of the sample and to eliminate outlier values through the Dixon-Reed analysis and Tukey Test. The method used was parametric with a robust method and a confidence level of 90% (Geffré *et al.*, 2011; Friedrichs *et al.*, 2012). Through this, it was found that the sample distribution was normal according to the Anderson-Darling normalcy test, with histograms and Q-Q plots. The central tendency measures were extracted (mean, median, standard deviation), maximum and minimum value and distribution charts. The statistical method for test hypothesis was used to compare the results found in this study with the reference values in the blood count to more than 3 000 meters masl performed in an earlier study in the Sierra central north of Ecuador. Similarly, the "Z normalcy test" was used for means of two samples, as it was a sample higher than 30 individuals and with normal distribution (Rodríguez, Gutiérrez y Pozo, 2007). A confidence level of 95% and a Z-critical point of 1.96 were set to accept or reject the study hypothesis (Dawson-Saunders y Trapp, 2005).

2.3 Specific handling methods of the experiment

It is very important to maintain a correct protocol for the results of the blood tests to be valid (Bolger, 2010). The blood samples were collected with animals at rest, before performing any type of physical activity and under conditions that minimized any likelihood of causing fear or arousal; this minimized the effect of splenic contraction (which would elevate the values of hematocrit, erythrocyte count and hemoglobin concentration), and the release of adrenaline or corticosteroids (which would cause modifications in the leucogram) (Cuenca Valera y Pastor Milán, 2006).

Prior to sampling, horses were kept moored at rest, in a quiet and undisturbed place for approximately 30 minutes, with the aim of avoiding possible alterations in their blood composition caused by physical activity or stress (Castillo, 2011). The samples were transported to the laboratory in a maximum time period of 24 hours after their extraction, and remained refrigerated at 4 °C. (Nuñez y Bouda, 2007). Prior to being analyzed blood samples should be homogenized by the self-hematology equipment Mindray®BC2800V (Mindray, 2012).

2.3.1 Universe and sampling

(Friedrichs *et al.*, 2012).

INEC (2012) states that in Ecuador there are 338 000 heads of horses, occupying the region Sierra the first place with 47.66%, followed by the Costa region with 36.69%, the Amazon region with 15.28% and the island region with 0.37%. As for the provinces of the coast region Manabi leads with 46 218, followed by Guayas with 31 942, emeralds with 22 782, Los Ríos con 12 493, El Oro with 6 459 and Santa Elena with 4 104 heads of horses. It is known that the majority belongs to horses with a non-specific breed, i.e, creole horses. However, there is currently no real census of the horse-herding population in the country. In the absence of current official figures on the horse population in Ecuador, it is decided to establish the sampling framework based on the guidelines dictated by the "American Association of Veterinary Clinical Pathologists" (ASVCP), in which is indicated that a sample size of $40 \leq x < 120$ can be used with a robust-parametric method and a confidence level of the reference limits of 90%

3 Results and discussion

Of the 100 samples taken and processed, a total of 19 samples were reported as potential outlier values for each analyte, from which 13 were eliminated since they corresponded to outlier data for the study of each analyte (see Table 1). The ages of the horses that were part of the study showed a minimum and maximum range of 2 and 20 years, with an average of 7.6 years and a standard deviation of $4.4 \pm$ (Figure 1). The results obtained from the hematological values of the horses born or reared between 0 and 500 masl are presented in the Ecuadorian Littoral region, with their respective central tendency measures (Table 2) and the distribution charts of the hematological values, represented as a straight line, which expresses the normality of the data obtained (Figure 2).

Table 1. Outlier values for each analyte under study

Analite	# of utlier	Final number of samples used to determine hematological values
Erythrocytes	1	100
Hemoglobin	3*	97
Hematocrite	0	100
VCM	0	100
HCM	0	100
CHCM	2	100
Leukocytes	2*	98
Lym#	2*	98
Mon#	3	100
Gran#	6*	94
Platelets	0	100

VCM: medium corpuscular volume

HCM: mean corpuscular hemoglobin

CHCM: mean corpuscular concentration hemoglobin

* values excluded from the study

Table 2. Hematological values of horses born or reared between 0 and 500 masl in the coastal region of Ecuador

Parameter	Unit	Mean	Median	SD	Minimum	Maximum
Erythrocytes	$10^6/\mu\text{L}$	6,96	6,84	1,07	4,9	9,38
Hemoglobine	g/dL	11,46	11,3	1,58	8,59	14,87
Hematocrite	%	33,86	33,15	4,97	24,83	45,1
VCM	fL	48,86	48,55	3,15	42,35	55,19
HCM	pg	16,52	16,6	0,98	14,25	18,2
CHCM	g/dL	33,95	33,9	1,17	32,1	36,7
Leukocytes	$10^3/\mu\text{L}$	9,16	9	1,84	5,64	12,81
Lym#	$10^3/\mu\text{L}$	3,24	3,1	1,19	1,04	5,85
Mon#	$10^3/\mu\text{L}$	0,51	0,5	0,17	0,2	0,9
Gran#	$10^3/\mu\text{L}$	5,12	5,05	1,44	2,9	8,26
Platelets	$10^3/\mu\text{L}$	198,75	201	56,02	78,1	314,9

SD: standard deviation

VCM: medium corpuscular volume

HCM: mean corpuscular hemoglobin

CHCM: mean corpuscular concentration hemoglobin

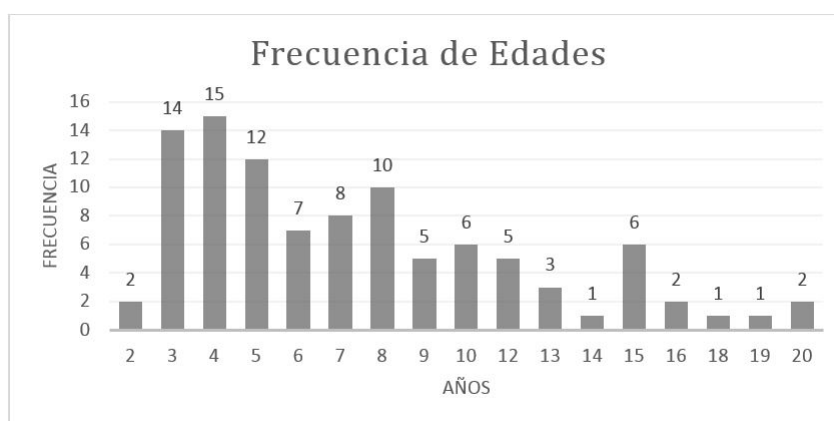


Figure 1. Age Frequency of the sampled horses to determine the hematologic range references.

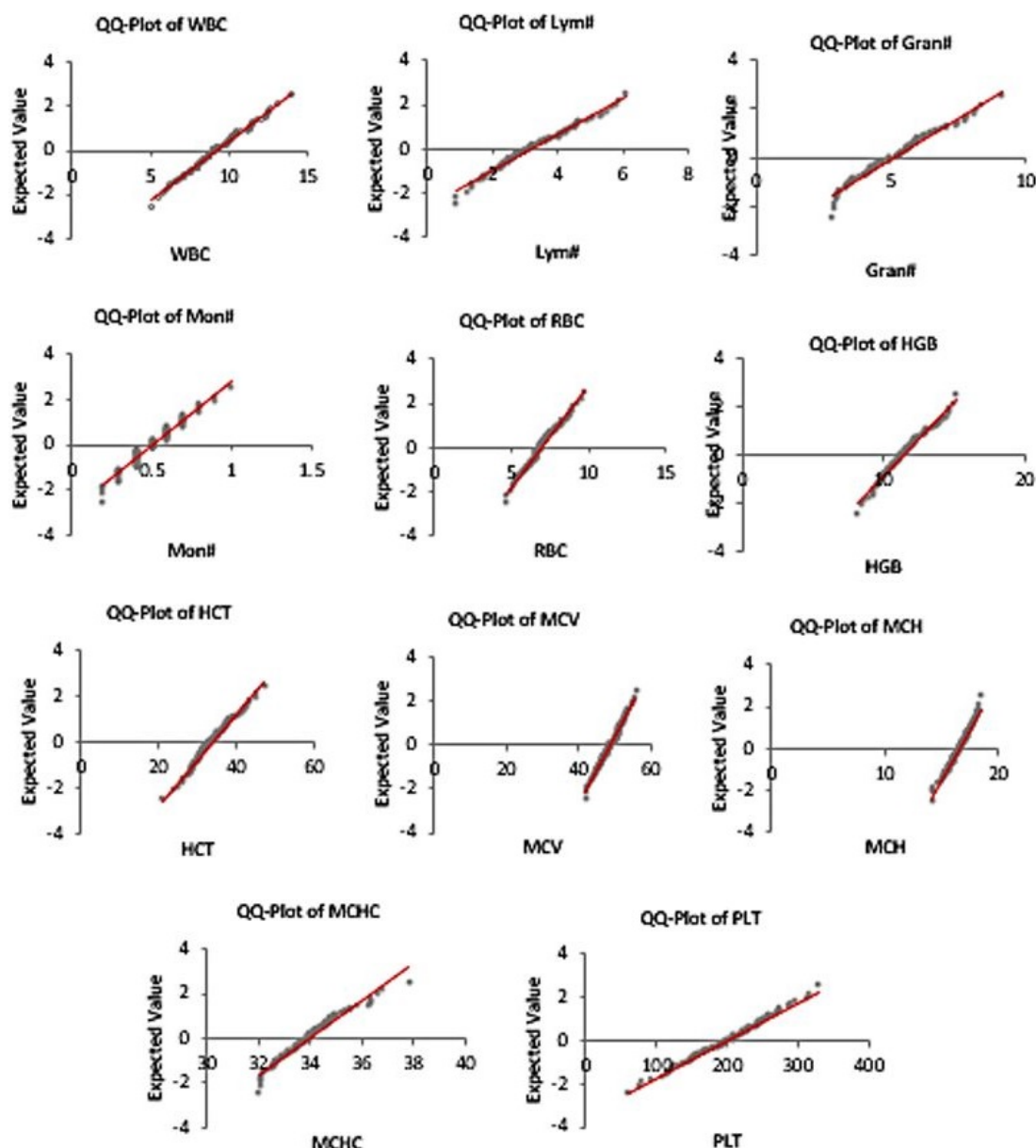


Figure 2. QQ-Plot distribution of each blood parameter ■: data, ■: normal distribution.

3.1 Comparison with the reported results

The results obtained in this study were compared with the results of the previous study conducted by Izurieta Barzola *et al.* (2017) (see Table 3).

When analyzing Table 3, where the results are compared with those obtained by Izurieta Barzo-

la *et al.* (2017) in the central north Ecuadorian Sierra, the reference values of erythrocytes, hemoglobin and hematocrit found in horses born or reared between 0 and 500 masl showed lower values in relation to the reference values in horses at more than 3 000 masl, showing a significant difference, which could be attributed to the variation in the altitude.

Table 3. Reference values and Z-test of Creole horses between 0 and 500 masl compared to the reference of Creole horses to more than 3 000 masl of Izurieta Barzola *et al.* (2017).

Parameter	Creole horses between 0 y 500 m.a.s.l.			Creole horses at more than 3000 m.a.s.l.			Z test
	Mean	Min-Max	SD	Mean	Min-Max	SD	
Erythrocytes	6,96	4,90-9,38	1,07	8,3	6,23-10,84	1	-8,71
Hemoglobine	11,46	8,59-14,87	1,58	14,1	11,4-18,4	1,6	-11,06
Hematocrite	33,86	24,83-45,10	4,97	41	32,3-52,2	4,4	-10,27
VCM	48,86	42,35-55,19	3,15	49,6	40,2-57	3,4	-1,51
HCM	16,52	14,25-18,20	0,98	16,9	14-20,3	1,1	-2,43
CHCM	33,95	32,10-36,70	1,17	34,3	32-37	1	-2,18
Leukocytes	9,16	5,64-12,81	1,84	8,3	4,8-12	1,7	3,26
Lym#	3,24	1,04-5,85	1,19	3,6	1,4-7,5	1,2	-2,01
Mon#	0,51	0,20-0,90	0,17	0,4	0,2-0,7	0,1	5,43
Gran#	5,12	2,90-8,26	1,44	4,3	2,2-8,8	1,4	3,82
Platelets	198,75	78,10-314,90	56,02	239	101-401	68,2	-4,29

SD: Standard deviation

VCM: Medium corpuscular volume

HCM: Middle corpuscular hemoglobin

CHCM: medium corpuscular Hemoglobin concentration

The study of Izurieta Barzola *et al.* (2017), also had significant differences with the values of erythrocyte count in Chilean mestizo horses, carried out in the region of Valdivia, being of $6.98 \times 10^6/\mu\text{L}$ DS 0.80 Böhmwald, Wegmann y Witter (1986). Also, with the hemoglobin values in the investigation of hematological values, bilirubin and serum enzymatic activity in Peruvian horses passing the valley of Lurín being the value of 13.90 g/dL DS 1.50 (Díaz *et al.*, 2011) and with the hematocrit value in an article conducted in Italy, with a value of 37.45% DS 4.97 (Giordano *et al.*, 2008). The results obtained in the three previously mentioned studies were carried out at sea level and with breeds of horses of warm blood of similar characteristics to the Ecuadorian Creole horse.

For an organism to adapt to different altitudinal floors, it must develop certain homeostatic changes, mainly in the cardiovascular, respiratory and hematologic systems. The mechanisms by which the adaptation to a higher height are produced are: increase of the respiratory rate, increase of the rate pulmonary ventilation, increase of the pulmonary diffusion capacity, increase of the vascularization of the peripheral tissues, increased number of erythrocytes and increased capacity of tissue cells to use available oxygen despite low oxygen pressure, all this in order for the organism to increase its ca-

capacity to capture and mobilize air and atmospheric oxygen despite the different environmental conditions (González Rengifo, 2001; Cárdenas, 20003; Hall, 2016). Life in the different height environments is translated as a situation of greater energy demand for the organism, so that the animals that live in high altitudes, where the atmospheric pressure decreases and the amount of oxygen is very reduced, face a tissue hypoxia which favors the secretion of erythropoietin, which is a glycoprotein that stimulates the formation of erythrocytes, hemoglobin and hematocrit (Suarez, 2001; Barranco *et al.*, 2002; Uscamayta Quispe, 2007). It should be emphasized that both sampling groups have similar breed characteristics, since they belong to the same type of Ecuadorian creole horses with a common origin, but reared at different altitudinal floors.

The reference value of the average corpuscular volume found in the horses born or reared between 0 and 500 masl showed similar values to the reference values in horses at more than 3 000 masl, being the only value that did not present significant difference. This is because the erythrocytes volume of the horse is always kept within rigid limits despite the presence of diseases, so that an increase in hematocrit corresponds to an increase in the number of erythrocytes and not the increase in the volume of these (Cuenca Valera y Pastor Milán, 2006).

As regards the reference values of the white count (leukocytes, lymphocytes, monocytes and granulocytes) determined in the horses born or reared between 0 and 500 masl, in some cases they showed slightly higher values and in others slightly lower in relation with the reference values in horses at more than 3000 masl, showing a significant difference. This may be due to different physiological, pathological stimuli, physical exertion, parasitic diseases, among others; but not a direct relation to the effect with the different altitudinal floors to which the animals can be subjected to (Navia *et al.*, 2004; Monroy, 2009).

In the reference value determined for platelets in horses born or reared between 0 and 500 masl with respect to the reference value in horses at more than 3 000 masl, there was significant difference since the value of animals at sea level is lower than the one of individuals living in high altitudes. Some authors such as Barbany (2002) point out that this can be attributed to the fact that animals in high places perform a more physical effort similar to intense exercise, due to the low amount of oxygen available, resulting in an increase in the platelet value. The normal platelets ranges in horses are varied (Cowell y Tyler, 2002). There may be a transient increase in platelet count that is released from its storage sources during a moderate muscle excitation activity (Weiss y Wardrop, 2010).

4 Conclusions

The data in this study have a normal distribution, which allowed to establish the reference values for clinically healthy Creole horses born or reared between 0 – 500 masl on the Ecuadorian coast, which will serve to be used in future studies and mainly in the equine clinic. When comparing the results obtained in the present study with the reference values of a previous study carried out at more than 3 000 masl in the Ecuadorian Central North Sierra, it was determined that there is a significant difference in the increase in the values of: erythrocytes, hemoglobin and hematocrit, which is due specifically to the variation effect of the altitude and not due to the type of horse, since in the two investigations were sampled creole animals, which originated in animals introduced during the Spanish conquest. Significant differences were found when comparing the white count values obtained in the present study with those

obtained at more than 3 000 masl, yielding both higher and lower values; so it is stated that this difference is not due to the altitudinal effect, but other causes that may be physiological or pathological influences. Platelets express lower values compared to the results found at 3 000 masl, attributed to the low oxygen amount available at high altitudes.

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