Reference Values for Some Hematological Parameters in Iraqi Local Horses

Hayder Dawood Saleem and Firas Rashad Al-Samarai*

Department of Veterinary Public Health, University of Baghdad, Iraq

ABSTRACT

A cross-sectional study was conducted to determine some hematological values in Iraqi local horses in order to increase the precision of diagnosis particularly no published data existed about this subject in Iraq. A total of 126 blood samples were collected from the jugular vein of horses randomly from several regions in the Middle and South of Iraq during the period from February to December 2017. The age of horses ranged from 2-17 year for both sexes (33 male and 93 female). Reference value adviser was used to estimates these parameters. Results revealed that the local reference intervals (LRI) as following: The red blood cell count (RBC) (5.61-$7.90 \times 10^6 \mu l$), hemoglobin (Hb) (8.42-$12.10 g/dL$), packed cell volume (PCV) (23.86-33.12%), corpuscular hemoglobin concentration (MCHC) (32.40-$41.30 g/dl$), corpuscular hemoglobin (MCH) (14.90-$16.90 pg$), corpuscular volume (MCV) (38.90-$52.00 fl$), and white blood cells (WBC) (6.78-$12.79 \times 10^3 \mu l$). The results showed that the lower limit of the LRI was lowered than the lower limit of the general reference intervals (GRI) for each of RBC, Hb, and PCV, while the lower limit of the LRI in the others parameters are within the range of the GI. The results obtained from statistical analysis confirmed that the effect of age was not significant, but the effect of sex was significant ($P<0.05$) only on PCV and Hb. The mean value of males was significantly ($P<0.05$) higher than females. In conclusion, the results of the LRI showed substantial differences as compared with the GRI and this will increase the need for establishing reference values for horses as well as for all local animals.

KEYWORDS: Reference values; Hematological parameters; Reference value advisor; Iraqi local horses

ABBREVIATIONS: Hb: Hemoglobin; RBC: Red Blood Cells; PCV: Packed Cell Volume; WBC: White Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RI: Reference Interval; LSD: Least Significant Difference; LRI: Local Horse Reference Intervals; GRI: General Reference Intervals

INTRODUCTION

The evaluating and monitoring the health status, the nutritional level and metabolic conditions of the animals can be performed based on the blood parameters profile [1].

The importance of the reference values lies in its role in the decision-making process related to the health status of the animal [2]. However, numerous studies confirmed that the hematological parameters influenced by some factors for instance: age, gender, breed, level of nutrition, type of activity and physiological status [3-6] this will not reduce its importance in the monitoring of the health, nutritional, and therapeutic status of horses.

The physical fitness and performance of horses depend on their health status, thus to achieve a high performance, it is very

important to maintain and control the health status of horses and this can be done by using reliable tools such as hematological parameters [6]. Several studies conducted to investigate the effect of age and gender on the reference values: for instance, Lana found a statistically significant effect of sex on Hb whereas no significant difference associated with age was detected in the Croatian cold blood horses. The significant high values of Hb and PCV are common in males [7] This could be attributed to the effect of androgens on erythropoiesis [8]. Similar results were obtained by Gordon et al., 2007 who reported that the variations in PCV according to age and gender were significant. On the other hand, Tomenendalova et al. [5]; Cruz et al. [9] confirmed the non-significant effect of age and gender on the Hb, PCV, RBC, MCHC, and WBC in horses.

The reference values of Iraqi local horses are nearly completely lacking. Moreover, a few researches have been conducted in Iraq included a small sample size [10,11], therefore, the reliability of these estimations was very low beside the estimations were limited to just of the range or/and the mean of the hematological parameters and this led to an inaccurate estimations as compared with the percentile confidence interval of LRI. Hence, this study was carried out to establishing reference values of Iraqi horses to be a useful guide for veterinarians to monitor the health status of animals along with to investigate the effect of age and gender on studied parameters.

MATERIALS AND METHODS

Study Design

A cross-section study was conducted on Iraqi local horses during the period from February to December 2017. The area of the survey included Baghdad, Waset, Al-Dewanai, Diyala, Babylon, AL-Najaf, Karbala and AL-Nasiriyah. The age of horses ranged from 2-17 year for both sex.

Animals

A total of 126 clinically healthy Iraqi local horses (33 male and 93 female) were selected randomly. In the sampling day, the breeders have been asked some questions about the current and past health status, sexual status, medications after that all animals subjected to complete physical examinations which were performed by an experienced veterinarian to ensure the health status.

Blood Samples

The blood sample (5ml) was drawn from the jugular vein after sterilizing the puncture area with 70% alcohol using disposable syringes. Blood samples were placed into labeled sterile tubes containing anti-coagulant (EDTA) and kept at 4 °C for hematological analyses which were performed within the next 24h. The blood samples were analyzed in the laboratory of the College of Veterinary Medicine/University of Baghdad/Iraq.

Hematological Parameters

The parameters used in the current study included: the red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hb), white blood cell count(WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The MCV, MCH, and MCHC were calculated according to Patterson et al. [12].

Statistical Analysis

The International Federation of Clinical Chemistry (IFCC) 1987 recommended using not less than 120 animals to determine the population-based reference interval (RI). Hence, data of 126 local horses were subjected to analysis to determine the reference values by using Reference Values Advisor V 2.1.

The data were analyzed by using SAS [13] to investigate the effect of the age and gender of the animal using the following mathematical model:

$$Y_{ijk} = \mu + A_i + G_j + e_{ijk}$$

Where:

Y_{ijk} is the studied trait

μ is the overall means

A_i the effect of ith age (i = ≤3, >3-6, >6-10, >10)

Gj the effect of jth sex (j = male, female)

e_{ijk} is the random error

Means were compared using the least significant difference (LSD). P<0.05 is considered statistically significant. Because there was a significant difference in Hb and PCV between males and females, the data were analyzed to estimate the reference interval for the two parameters of each sex.

RESULTS

Table 1: The reference intervals of some hematological parameters in clinically healthy Iraqi local horses estimated by the reference value advisor.

Parameter	Unit	Mean	Median	SD	Lower limit RI	LL(CI$_{90\%}$)	Upper limit RI	UL(CI$_{90\%}$)	Method	GRI
RBC	x10^6	6.52	6.4	0.57	5.61	(5.51-5.77)	7.9	(7.83-7.90)	NP	6.8-12.9
Hb	g/dL	10.24	10.2	0.89	8.42	(8.20-8.70)	12.1	(12.00-12.10)	NP	19-Nov
PCV	%	28.49	28.3	2.33	23.86	23.33-24.42	33.12	32.52-33.69	S	32-53
MCHC	g/dl	36.62	35.95	2.67	32.4	(23.40-33.00)	41.3	(41.20-42.00)	NP	31.0-38.6
MCH	pg	15.89	15.9	0.54	14.9	(14.80-1510)	16.9	(16.80-17.10)	NP	12.3-19.7
MCV	fl	43.98	44.3	3.82	38.9	(38.90-38.90)	52	(51.00-54.00)	NP	37-59
WBC	x10^3	9.78	9.85	1.51	6.78	(6.43-7.14)	12.79	(12.41-13.15)	S	5.4-14.3

GRI: General reference values (Constable et al., 2016)

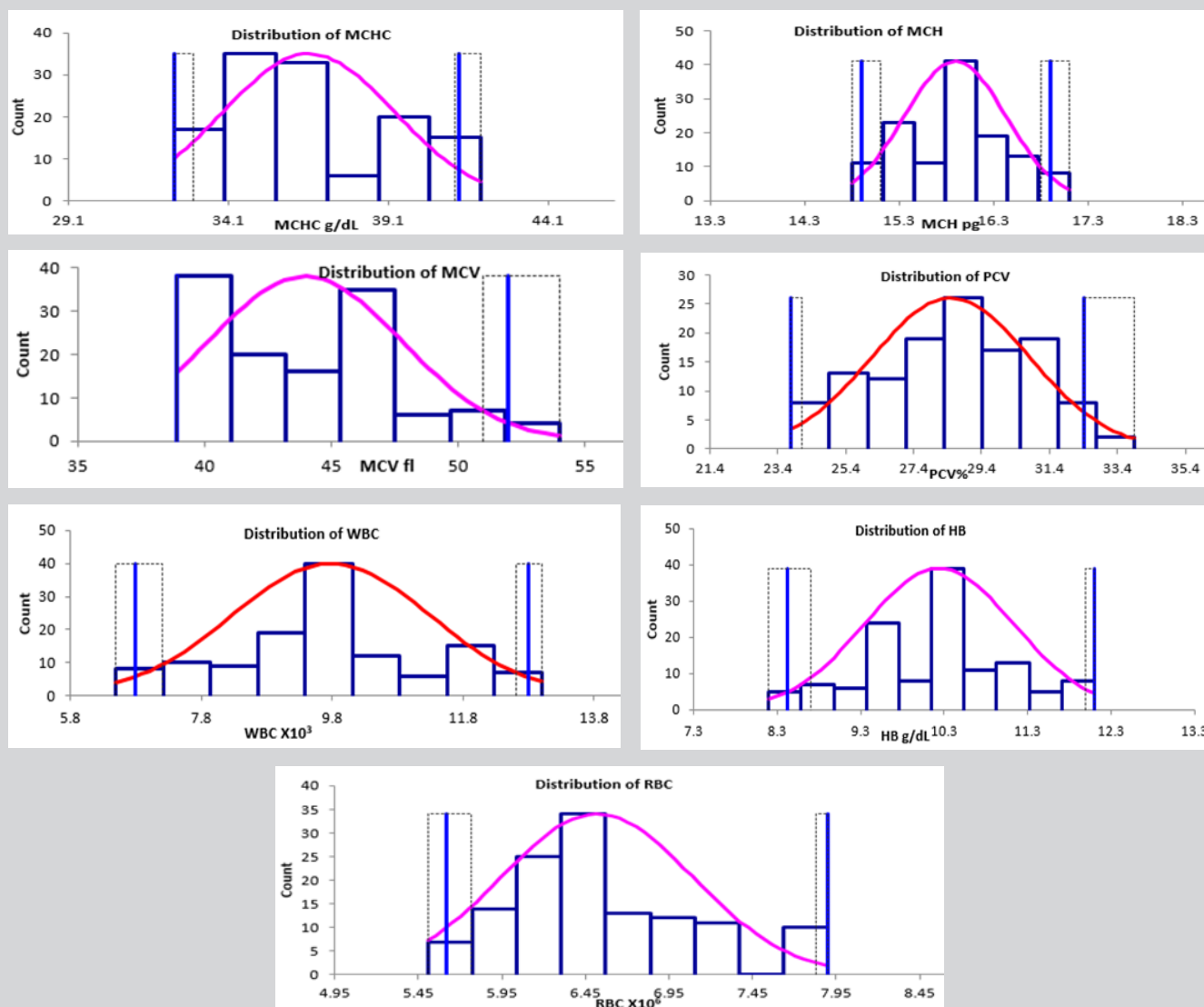NP: Non-parametric method

S: Standard method

Figure 1: Distributions of observed (blue boxes) and fitted (purple lines) curves of some hematological parameters of 126 clinically healthy Iraqi local horses. Blue vertical lines are the reference limits with corresponding 90% confidence intervals as the dotted line. Graphs with purple curve were not normally distributed whereas graphs with the red curve are normally distributed.

The results of hematological local horse reference intervals (LRI) are shown in Table 1 which was also illustrated the general reference intervals (GRI) [14]. The distribution of all parameters is shown in the Figure 1. The data of PCV and WBC were normally distributed; therefore, we have selected the standard method for the estimation of the reference values (Figure 1). The distribution of the data of the others parameters did not follow the normal distribution and the test of Anderson-Darling confirmed that the standard, Box-Cox standard, Robust, and Robust Box-Cox are not suitable for estimation reference values, thus in such case the non-parametric test is the last solution. The 95% Lower and Upper limit of Hb in Iraqi horses (8.42-12.10g/dl) are lowest than the corresponding estimations of the GRI [3,4,7,11-13,15-17]. The situation is the same in PCV (23.86-33.12%) and RBC (5.61-7.90x 10^6). The corresponding estimations of the GRI are 32-53% and 6.8-12.9x10^6. Concerning to the estimations of the MCV (38.90-52.00fl), MCH (14.90-16.90pg) and WBC (6.78-12.79x10^3), these estimations are within the range of GRI. For the MCHC (32.40-41.30 g/dl) the upper limit was higher than the upper limit of the both GRI.

The statistical analysis showed no significant effect of age on all parameters (Table 2), whereas the effect of sex was significant (P<0.05) on PCV and Hb (Table 3). The estimations in males (28.84%, 10.48g/dl) were higher than females (27.20%, 10.01g/dl). Hence, the data of males and females were subjected to analysis to establish reference values of Hb and PCV for each sex (Table 4). The lower and upper RI of male was 8.39-12.37g/dL and 22.69-38.18% for Hb and PCV respectively while the corresponding estimations in females were 7.20-12.10g/dL and 17.65-33.31%.

DISCUSSION

Despite the importance of hematological reference values of equines, there are a few reports of Iraqi local horses which were published, and the estimations of values are usually presented as the range (lowest and highest values) or as the mean ± standard deviation. Thus, this research represents in our knowledge the first work to establish the reference values for Iraqi local horses which are virtually absent in Iraq. The determination of the reference values was performed using reference value advisor and the values

were presented as lower and upper limits. Therefore, these values will be more accurate for Iraqi local horses than the previously published values presented as mean and range and more suitable than the general reference values because these values reflect the real values of Iraqi local horse under Iraqi local environment.

However, the reference values of Iraqi local sheep [18] and goats [19] using reference values adviser were published recently, but there is a lack of reference values for the most Iraqi local animals such as cows, buffaloes, camels, and donkeys along with all the poultry.

Table 2: The effect of age on some hematological parameters in Iraqi local horses.

Age/yr.	No	RBCx10⁶	PCV%	Hb g/dl	MCV fl	MCH pg	MCHC g/dl	WBCx10³
≤3	20	6.35±0.17	27.78±0.67	10.26±0.26	44.41±0.86	15.84±0.13	36.14±0.58	9.23±0.41
>3-6	30	6.24±0.17	26.68±0.89	9.80±0.26	43.61±0.69	15.91±0.08	37.01±0.47	9.96±0.27
>6-10	39	6.52±0.13	28.16±0.67	10.37±0.18	44.34±0.62	15.92±0.08	36.22±0.44	9.54±0.28
>10	37	6.36±0.10	27.75±0.71	10.08±0.14	43.64±0.63	15.88±0.09	36.95±0.43	9.93±0.30

All differences are not significant

Table 3: The effect of gender on some hematological parameters in Iraqi local horses.

Sex	No	RBCx10⁶	PCV%	Hb g/dl	MCV fl	MCH pg	MCHC g/dl	WBCx10³
Male	33	6.53±0.11	28.84±0.68a	10.48±0.16a	44.52±0.67	15.81±0.09	36.37±0.41	9.79±0.32
Female	93	6.32±0.08	27.20±0.42b	10.01±0.12b	43.78±0.39	15.92±0.05	36.70±0.28	9.68±0.17

Table 4: Reference intervals of Hb and PCV in the Iraqi local horses according to sex.

Parameter	Unit	Mean	Median	SD	Lower limit RI	LL(CI$_{90\%}$)	Upper limit RI	UL(CI$_{90\%}$)	Method
Hb	g/dL	10.48	10.3	0.94	8.39	7.39-8.91	12.37	11.76-12.90	R
Male									
Female		10.01	10.2	1.23	7.2	7.20-7.34	12.1	11.70-13.00	NP
PCV	%	28.85	28.4	3.95	22.69	21.21-24.54	38.18	34.66-42.17	BCR
Male									
Female		27.2	28.3	4.1	17.65	17.40-18.74	33.31	32.05-33.90	NP

R=Robust

NP=Non-parametric

BCR=Box-Cox Robust

The results of hematological parameters showed that only the PCV and WBC are normally distributed, and the other parameters did not normally distribute. Several researches reported that the blood parameters are most often not normally distributed in animals [6,19,20]. Therefore, the appropriate method for estimating the reference interval is the non-parametric method as this method did not need any specific distribution of the data [21]. All references values showed lower width between lower and upper limits as compared with GRI. These results are expected since the values of GRI included different breeds, management, and climates [6,17]. Also, results showed the lower limit of RBC, Hb, and PCV was lowered than the lower limit of GRI. The differences between LRI and GRI could be attributed to the adaptation of animals to the harsh environment and the way of breeding. The significant ($p<0.05$) differences in the Hb and PCV due to sex in a current study was agreed with results obtained by Gupta et al. [3], Lana and Tomenendalova et al. [5]. Also, the non-significant effect of age on all parameters was observed in this study which is in the line with the results reported by Lana However, there were some studies showed a significant effect of age on hematological parameters [3,5,9]. These differences among studies could be reflecting the difference in the sample size, breed, and climate [22].

CONCLUSION

The results of the present study confirmed the need of establishing reference values for Iraqi local horses as there was a considerable difference between LRI and GRI ant these differences could be attributed to the adaptation of animals to the harsh environment and the way of breeding.

ACKNOWLEDGMENT

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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