

Haematological parameters in a free-ranging population of *Didelphis virginiana* from Mexico

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ABSTRACT. The American opossum *Didelphis virginiana* is the marsupial species with the largest geographic distribution in North America, a very important natural host and key to the maintenance and transmission of many zoonotic pathogenic microorganisms of importance in public health, and one of the wild mammals with the greatest adaptation to the human environment, but despite all that very little is known about some physiological aspects in their free-ranging populations. In the present study, basic haematological parameters of 201 opossums from a synanthropic population of *D. virginiana* in a rural locality of the state of Yucatan, are first described. The average values of haemoglobin, packed cell volume, red blood cells, and eosinophils were higher in males and adults (except eosinophils) than in females and juveniles, respectively, and juvenile opossums had higher values of lymphocytes, neutrophils, and platelets than males. Non-pregnant females had significantly higher values of mean corpuscular volume, lymphocytes and platelets than pregnant ones, while red blood cell count and segmented neutrophils showed higher values in pregnant females. The establishment of the basic haematological parameters for free-ranging populations of *D. virginiana* is a very useful reference for both the health monitoring of the populations and further studying the host-parasite relationship of some zoonotic pathogens present in the Yucatan Peninsula.

Key words: synanthropic *Didelphis virginiana*, haematology, Mexico, opossum, Yucatan.

INTRODUCTION

The opossum *Didelphis virginiana* is the marsupial with the largest geographic distribution in North America, particularly in Mexico (Gardner 1973). According to Ruiz-Piña *et al* (2013), the current populations of *D. virginiana* in Mexico are commonly associated with human housing and are identified as synanthropic. Opossums are recognised as hosts of numerous pathogenic microorganisms for humans, and responsible of maintenance of the transmission cycle of many of them (Jansen *et al* 1991, Travi *et al* 1994, Boostrom *et al* 2002). In Mexico, the presence of zoonotic pathogens such as parasites (García-Márquez *et al* 2010, Acosta-Virgen *et al* 2015, Torres-Castro *et al* 2016), bacteria (Ruiz-Piña *et al* 2002, Peniche-Lara *et al* 2016), and viruses (Ruiz-Piña *et al* 2010), have been documented for *D. virginiana*. In this context, the systematic collection and monitoring of haematological values of free-ranging populations of wild or synanthropic animals can provide baseline information to determine their natural physiological status or the diagnosis of their own diseases or associated with contact with human populations (Stevens *et al* 2012). To date, published studies on haematological parameters of *D. virginiana* has been carried out on animals in captivity or with very few individuals from natural populations (Lewis 1975); haematological parameters are not currently available for

wild/free-ranging *D. virginiana* in Mexico. Considering all the above, the objective of this work was to describe the first data in Mexico on hematological parameters of *D. virginiana* from a rural synanthropic population of Yucatan state, in order to generate basic information about the physiological status of this mammal, which can subsequently be used in future studies in the region as the impact of diseases in their natural populations, the ecology of their host-parasite interactions and their involvement in the transmission cycles of zoonotic pathogens.

MATERIAL AND METHODS

The study was conducted in the rural town of Molas, from the municipality of Merida, Yucatan, Mexico from December 2009 to December 2014. Molas is located 12 km southeast of the city of Merida city (20° 49' 39" N and 89° 37' 36" W), at an altitude of 10 meters above sea level. The predominant climate is warm sub-humid with summer rains and an average annual temperature of 26 °C (Orellana *et al* 2010). The predominant vegetation in the surroundings of the locality is of low deciduous forest (Flores and Espejel 1994).

Opossums were captured during three consecutive nights per month in the backyards of 40 households between October 2009-April 2010, October 2011-December 2012, and January-December 2014 (Approval of the Bioethics Committee: CIE05-08). A Tomahawk® trap baited with fresh fruits was placed before sunset in each backyard. The captured animals were collected as early as possible the following morning. A total of 201 individuals of *Didelphis virginiana* were captured of which 115 were females and 86 males, 146 adults and 55 juveniles. Forty-two females were virgin and 24 were pregnant.

Received: 14.10.2018.

Accepted: 08.07.2019.

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The identification of *D. virginiana* was carried out using the taxonomic keys of Gardner (1973) and Hall (1981).

Captured opossums were transported to the laboratory, and kept in the quarantine area for a maximum of 24 hr before their release back to the capture locality. Captive opossums were fed with water *ad libitum*, fresh fruits and cat food. Housing conditions and management of captured animals followed the recommendations of Jurgelski (1974), Gannon and Sikes (2007) and complied with NOM-062-ZOO-1999. All applicable institutional and/or national guidelines for the care and use of animals were followed.

Somatic data and the dental formula were taken from opossums for the determination of age (adult and juvenile) (Petrides 1949). Reproductive status of females was determined according to Reynolds (1952) as follows: virgin (short and abundant whitish hair, and imperceptible small nipples) and pregnant (presence of embryos in the marsupium).

Blood samples of 0.5 to 1 mL were obtained from the caudal vein of opossums, deposited in Microtainer® tubes with EDTA, and kept at room temperature until processing within a period of no more than 30 minutes. The blood samples were gently mixed before insertion into the automatic analyser SISMEM KX 21N (Roche®) to obtain Red Blood Cells Count (RBC), Packed Cell Volume (PCV), Haemoglobin (HGB), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), and White Blood Cells Count (WBC). The differential leukocyte count was performed manually to observe the leukocyte, red blood cell, and platelet count and morphology. The differential count of leukocytes was performed on blood smears stained with Wright's dye. Based on the count of 100 cells per smear using a microscope (Nikon®), the following leukocyte parameters were estimated: Lymphocytes (LI), Monocytes (MO), Eosinophils (EO), Basophils (BA), Segmented Neutrophils (SN), and Platelets (PLT).

Descriptive statistics (mean \pm standard deviation) were used for statistical comparisons of the parameters. Reference limits and 90% confidence intervals were calculated for every parameter using the Reference Value Advisor freeware (Geffré *et al* 2009) according to Friedrichs *et al* (2012). Mann-Whitney U test was used because some parameters were not normally distributed or lack of homogeneity of variance was observed. The comparisons were performed with R software version 3.4.4 (2018). The level of error allowed in the contrasts was 5% (Zar 2010).

RESULTS AND DISCUSSION

The values of the haematological parameters obtained in this study are presented in the tables below, which show comparisons of haematological values between sex (table 1), age (table 2), and reproductive status (table 3).

Table 1 shows that HGB, PCV and RBC, were higher in males ($P < 0.05$). Also, it can be observed that adults

had greater values of PCV, RBC, and SN than juveniles ($P < 0.05$), however, MCHC, MCV and LI were significantly higher in juveniles ($P < 0.05$) (table 2). Virgin females had significantly higher values of MCV, LI and PLT than pregnant ones, although the RBC and SE parameters were higher in the later ($P < 0.05$) (table 3).

This study reports the first description of haematological parameters in a free-ranging population of *D. virginiana* associated with backyards of human households. Previous reports on haematological parameters of this species were made from individuals kept in captivity (Giacometti *et al* 1972, Mays and Loew 1968, Timmons and Marques 1969). Although Lewis (1975) included free-ranging opossums in his haematological study and the results were similar to those found in this study, comparisons should be made with many reservations, because that study included animals purchased from a biological product store and were kept in captivity several days before blood analysis, with blood sample being taken from the heart. Therefore, due to differences in the management protocols, and the physiological status of the opossums, our results will not be compared with previous publications on the subject. Instead, we will briefly discuss those differences found in the haematological parameters of *D. virginiana* related to developmental conditions (age and reproductive status) and sex, which are commonly related to mammal physiology. In this sense, the higher values of RBC, PCV, and HB in males can be explained by the body mass of opossum males in relation to females (Promislow 1991), in addition, the difference of some haematological parameters such as HGB and PCV found between sexes is common among vertebrates and particularly among mammals (including marsupials), and it has been directly related to the sex hormones, both estrogen and androgens on erythropoiesis (Murphy 2014, Barnes *et al* 2008). The same differences regarding those parameters were found in pregnant females when compared to virgin females (Sealander 1964, Murphy 2014, Nse Abasi *et al* 2014). The higher values of lymphocytes and platelets in juveniles, in addition with higher concentrations of haemoglobin and greater mean globular volume in comparison with adults, is something expected for individuals of this age class in both domestic in wildlife mammals (Campbell 2015, Thrall *et al* 2012, Wu *et al* 2014, Murphy *et al* 2014, Castañeda-Herrera *et al* 2015). An increase in age-related HGB and PCV concentrations have also been shown in common brush-tail possums (Presidente and Correa 1981).

On the other hand, pregnant females had higher values of mean corpuscular volume (MCV), but corpuscular haemoglobin (MCHC, CHC), and erythrocyte volume were lower than not pregnant females. This is generally the case because in mammals the need for iron in the mother increases during the lactation period, resulting in higher values of red blood cells in pregnant females, but some kind of anaemia is commonly observed (Campbell 2015, Guzman-Medina and Callacná-Custodio 2013). In

Table 1. Mean and standard deviation (SD) of the haematological parameters of *Didelphis virginiana* by sex.

Parameter	n	Female (SD)	LRL (90%CI)	URL (90%CI)	n	Male (SD)	LRL (90%CI)	URL (90%CI)	P
(units)									
HGB (g/dL)	111	11.8 (1.2)	9.5 (9.2-9.8)	14.1 (13.8-14.4)	83	12.8 (1.6)	9.6 (9.1-10.1)	16.1 (15.6-16.6)	<0.001
PCV (%)	112	40.4 (4.6)	31.4 (30.2-32.6)	49.5 (48.3-50.7)	80	43.3 (5.7)	32.8 (30.4-34.4)	56 (52.7-56.5)	<0.001
RBC (10 ⁹ /µL)	110	5.2 (0.6)	3.9 (3.8-4.1)	6.5 (6.3-6.7)	85	5.6 (0.9)	3.9 (3.6-4.1)	7.4 (7.1-7.6)	<0.001
MCHC (g/dL)	110	29.3 (1)	27.3 (27.1-27.6)	31.3 (31.1-31.6)	85	29.5 (1)	27.6 (27.3-27.9)	31.4 (31.1-31.7)	0.193
MCH (pg)	115	22.9 (1.8)	19.3 (18.8-19.8)	26.6 (26.1-27.1)	86	22.7 (1.6)	19.4 (19-19.9)	26 (25.6-26.6)	0.469
MCV (fL)	115	78 (4.8)	68.4 (67.1-69.8)	87.5 (86.2-88.7)	85	76.9 (4.6)	67.6 (66-69)	86.2 (84.7-87.5)	0.11
WBC (µL)	114	17.8 (5.6)	6.6 (5-8.3)	29 (27.4-30.5)	84	17.5 (5.5)	8.5 (7.9-10.1)	29.3 (28.6-29.4)	0.447
LI (%)	115	59.7 (14.4)	31.1 (27.6-35)	88.3 (84.4-92.4)	84	60.5 (12.9)	31.5 (29-37)	83 (80.5-84)	0.739
MO (%)	113	1.1 (1.6)	0	5.3 (4-7)	86	1.1 (1.7)	0	6 (5-6)	0.549
EO (%)	114	6.1 (4.2)	0	16.1 (13.3-18)	83	4.8 (3.7)	0	13 (12-15)	0.032
BA (%)	113	0.4 (0.8)	0	3 (2-4)	84	0.5 (0.8)	0	2.9 (2-3)	0.626
SN (%)	115	30.1 (14.9)	3 (0-9.3)	61.1 (54.7-74)	86	30.8 (15.7)	1.4 (0-12)	72.4 (59.8-75)	0.858
PLT (10 ³ /µL)	109	492.6 (127.1)	218.8 (213-248)	727.5 (695.1-780)	83	484.4 (100.4)	283 (252.2-316.3)	685.3 (651.8-717.2)	0.337

LRL=lower reference limits, URL=upper reference limits 90% confidence intervals (90%CI). P=p-value from comparisons with Mann-Whitney test.

HGB=Haemoglobin, PCV=Packed Cell Volume, RBC=Red Blood Cells Count, MCHC=Mean Corpuscular Hemoglobin Concentration, MCH=Mean Corpuscular Hemoglobin, MCV=Mean Corpuscular Volume, WBC=White Blood Cells Count, LI=Lymphocytes, MO=Monocytes, EO=Eosinophils, BA=Basophils, SN=Segmented Neutrophils, PLT=Platelets.

Table 2. Mean and standard deviation (SD) of the haematological parameters of *Didelphis virginiana* by age.

Parameter	n	Adult (SD)	LRL (90%CI)	URL (90%CI)	n	Juvenile (SD)	LRL (90%CI)	URL (90%CI)	P
(units)									
HGB (g/dL)	144	12.3 (1.7)	9.4 (6.9-10.1)	16 (15.5-16.5)	55	11.7 (1.5)	6.5 (5.6-9.3)	14 (13.7-14)	0.051
PCV (%)	142	42.2 (5.8)	32 (24.2-34.2)	55.4 (52.2-59)	54	39.3 (4.7)	26.9 (25.5-32.8)	47.1 (46.8-47.1)	0.002
RBC (10 ⁹ /µL)	143	5.5 (0.8)	3.8 (3.3-4.3)	7.2 (7.1-7.4)	53	4.9 (0.6)	3.7 (3.5-4)	6.1 (5.9-6.4)	<0.001
MCHC (g/dL)	144	29.2 (1)	27.2 (26.3-27.6)	31.1 (30.7-31.6)	55	30.1 (1.1)	27.3 (27.1-28.2)	33 (31.6-33.5)	<0.001
MCH (pg)	146	22.4 (1.6)	18.9 (18.4-20)	25.4 (25-27.8)	55	23.9 (1.6)	20.7 (20.1-21.3)	27.2 (26.6-27.8)	<0.001
MCV (fL)	146	76.8 (4.7)	68.1 (66.3-69.9)	87.2 (85-91.3)	55	79.6 (4.6)	70.3 (68.5-72.2)	88.9 (87.1-90.7)	<0.001
WBC (µL)	143	17.7 (5.5)	8.3 (3.7-10.4)	29.2 (28.7-31.8)	55	17.6 (5.9)	5.8 (3.6-8)	29.5 (27.3-32)	0.761
LI (%)	146	58.1 (14.1)	30 (26.7-33.4)	86.1 (83-88.9)	51	66.6 (9.9)	46.5 (42.5-50.8)	86.6 (82.6-90.4)	<0.001
MO (%)	145	1.1 (1.5)	0	5 (4-6)	54	1.1 (1.9)	0	7 (5.3-7)	0.32
EO (%)	145	5.5 (4.3)	0	17 (15-20)	55	6.4 (4.1)	0	15.4 (12.6-17)	0.106
BA (%)	142	0.4 (0.7)	0	2.4 (2-3)	54	0.4 (0.8)	0	2.6 (2-3)	0.964
SN (%)	146	32 (15.2)	2 (0-10)	63.7 (59-74)	54	25.3 (13)	4.5 (3-10)	61.6 (48.1-62)	0.003
PLT (10 ³ /µL)	140	474 (117.9)	217.6 (188-248)	694.8 (659-780)	55	533.6 (121.7)	258 (256-349.4)	816 (720-838)	0.004

LRL=lower reference limits, URL=upper reference limits 90% confidence intervals (90%CI). P=p-value from comparisons with Mann-Whitney test.

HGB=Haemoglobin, PCV=Packed Cell Volume, RBC=Red Blood Cells Count, MCHC=Mean Corpuscular Hemoglobin Concentration, MCH=Mean Corpuscular Hemoglobin, MCV=Mean Corpuscular Volume, WBC=White Blood Cells Count, LI=Lymphocytes, MO=Monocytes, EO=Eosinophils, BA=Basophils, SN=Segmented Neutrophils, PLT=Platelets.

Table 3. Mean and standard deviation (SD) of the haematological parameters of female *Didelphis virginiana* by reproductive status.

Parameter (units)	n	Pregnant	LRL (90%CI)	URL (90%CI)	n	Non Pregnant	LRL (90%CI)	URL (90%CI)	P
HGB (g/dL)	23	11.8 (1.1)	9.4 (8.7-10.1)	14.2 (13.5-15)	41	11.7 (1.2)	9.3 (8.8-9.8)	14.1 (13.6-14.6)	1
PCV (%)	23	40.5 (4.8)	30.3 (27.1-33.2)	50.7 (47.5-53.4)	41	39.3 (4.3)	30.4 (28.6-32.6)	48.1 (46.2-50.2)	0.488
RBC (10 ⁶ /μL)	22	5.3 (0.6)	3.7 (3.3-4.1)	6.5 (5.9-7.1)	41	4.9 (0.6)	3.7 (3.4-4)	6.2 (5.9-6.4)	0.037
MCHC (g/dL)	24	29.3 (1.1)	27 (26.4-27.8)	31.5 (30.8-32.2)	42	29.9 (1.2)	27.1 (27.1-28.1)	33.4 (31.5-33.5)	0.022
MCH (pg)	24	22.5 (1.7)	19 (18-19.9)	26 (25-27)	42	24 (1.6)	20.2 (20.1-21.7)	27.6 (26.2-27.6)	0.001
MCV (fL)	24	76.7 (4.5)	67.2 (64.2-70.2)	86.3 (83.4-88.9)	42	80 (4.7)	70.5 (68.4-72.7)	89.5 (87.6-91.5)	0.007
WBC (μL)	23	16 (5.3)	4.6 (1.4-8.4)	27.3 (23.8-30.3)	42	18.2 (5.7)	4.4 (4.3-9.4)	30.4 (28-30.5)	0.053
LI (%)	24	55.3 (12.2)	29.4 (21.9-37.4)	81.1 (72.9-88.3)	40	67.5 (10.6)	45.8 (41-51.3)	89.1 (84.3-93.3)	<0.001
MO (%)	23	1 (1.2)	0	4	40	1 (1.3)	0	4	0.72
EO (%)	24	6 (4.6)	0	15.6 (12.7-18.3)	42	6.5 (3.7)	0	13 (12-13)	0.374
BA (%)	24	0.5 (1)	0	2.6 (1-3.8)	42	0.3 (0.6)	0	2 (1-2)	0.87
SN (%)	24	35.5 (12.7)	8.8 (1.1-17.4)	62.3 (54.1-69.5)	42	24.2 (14.1)	0.2 (0-7)	61.9 (49.2-62)	0.001
PLT (10 ³ /μL)	24	413.2 (179.5)	ND	ND	37	549.7 (83.8)	432.4 (421.6-447)	869.3 (707.6-1066.2)	<0.001

LRL=lower reference limits, URL=upper reference limits 90% confidence intervals (90%CI), P=p-value from comparisons with Mann-Whitney test.

HGB=Haemoglobin, PCV=Packed Cell Volume, RBC=Red Blood Cells Count, MCHC=Mean Corpuscular Hemoglobin Concentration, MCH=Mean Corpuscular Hemoglobin, MCV=Mean Corpuscular Volume, WBC=White Blood Cells Count, LI=Lymphocytes, MO=Monocytes, EO=Eosinophils, BA= Basophils, SN= Segmented Neutrophils, PLT= Platelets.

this regard, Barnes *et al* (2008) did not find a directly proportional relationship between the concentration of HGB with lactation demand in the Brush-Tailed Rock-Wallaby, an Australian marsupial.

The results of this study represent the first information of the haematological aspects of a population of opossums associated with the human peridomicile in Mexico. The haematologic values obtained reflect a natural condition of the population of opossums in the studied locality and probably in many populations of this species in the state of Yucatan. The results of this study do not allow us to associate the values obtained with any infection or disease present in the animals studied, but they are the basis for further investigations to be carried out by our research group, either to analyse the health status of the populations of opossums that coexist with human populations and its domestic fauna, or to research its role as reservoir-host of zoonotic pathogens in the Yucatan Peninsula (Parada-López *et al* 2013, Ruiz-Piña and Cruz-Reyes 2002, Ruiz-Piña and Reyes-Novelo 2012).

In conclusion, based on the sample size of the *D. virginiana* population studied, the results of the haematological parameters obtained are informative reference values for a natural synanthropic population of this species that are distributed in the studied tropical region.

ACKNOWLEDGEMENTS

The present study was supported by the project "Monitoring, Surveillance and Intervention of Zoonotic Pathogens and ETVs in a Rural Area of the State of Yucatan" of the Epidemiological Network of Zoonotic and Vector-Transmitted Diseases (CIRB-2013-0007-SEP/PROME). Special thanks to Alan D. Cuxim Koyoc and Rosendo A. Aragón Pech for their invaluable support in the fieldwork and laboratory.

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