

Anthropogenic Influence on Blood Biomarkers of Stress and Genotoxicity of the Burrowing Owl (*Athene Cunicularia*)

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Abstract

Anthropogenic activities are putting increasing pressure on ecosystems and raising the need to assess environmental health quickly and accurately. Heterophile/lymphocyte index is accepted as a response to stress factors and the micronucleus test is used as a biomarker to estimate genotoxic damage. To evaluate a model of environmental quality, healthy Burrowing owl (*Athene cunicularia*) were sampled, and the heterophile/lymphocyte ratio was determined, as well as the frequency of micronucleated erythrocytes and nuclear abnormalities, determined with the Wright-Giemsa and acridine orange technique. The sites with the greatest anthropic disturbance recorded the highest frequencies of micronuclei and heterophile/lymphocyte index. The combination of both tests allows the detection of possible acute or chronic exposure to stressors and genotoxic contaminants, both in healthy or altered ecosystems.

Keywords: Aguascalientes; Biomonitoring; Heterophile; Lymphocyte; Micronucleus; Nuclear abnormality; Owl

Introduction

Anthropogenic activities modify ecosystems, mainly due to habitat degradation, loss and pollution [1,2], which exposes the wild populations to stressors and pollutants causing them become vulnerable. To assess these effects, the heterophile/lymphocyte index (H/L index) was used as a measure of physiological response to immunosuppression and stress [3,4], caused by various etiological agents. A high H/L index consists of an increase in the frequency of heterophiles and a decrease in lymphocytes [5]. In the response to chronic stress, this index may be more useful and accessible than a single measurement of plasma corticosterone [6].

On the other hand, micronuclei can occur spontaneously, however, in the presence of certain endogenous or exogenous stresses [7,8] the frequency of MN increases, becoming indicators of the effects of genotoxicity [9]. Micronuclei (MN) and nuclear abnormality (AN) frequency can be used as biomarkers to estimate genotoxic damage caused by physical, chemical or biological agents [10,11]. In addition, its low cost and relative ease, makes the MN test suitable the guideline to be considered as a routine technique in environmental monitoring studies.

The Burrowing Owl (*Athene cunicularia hypugea*: Strigidae) lives mainly in prairie grasslands and open areas [12] of north America [13], which advantage the possibility of studying different spaces. It combines the ideal characteristics to be considered biomonitor of environmental genotoxics, is top predator of small size, with superior clutches to three offspring per season [14]. They are territorial birds that facilitate their location, are sheltered in or stay near burrows and develop activities both day and night [15]. Additionally, its handling is relatively easy and it tolerates a certain degree of disturbance of anthropic origin. However, some basic parameters of biomarkers are not known to propose as a model. The aim of this study was to calculate the abundance and determine the values of heterophile/lymphocyte index and micronuclei of Burrowing owl from three sites with different degree of disturbance.

Materials and Methods

Sampling was carried out in the south and northeastern of the state of Aguascalientes, Mexico (Figure 1). Into township Aguascalientes, El Llano and Asientos with an average altitude of 2000 m. The climate of the entity is semi-dry with an average annual temperature of 17.4°C and average rainfall of 526 mm. The native vegetation is represented by oak forests in some high parts, grass (with the dominant genera of *Aristida* sp., *Buchloe* sp., *Bouteloua* sp. and *Microchloa* sp.) and different kind of xerophilous scrub characterized by cacti as nopaleras [16-18]. In the last decade the industrial and mining investment has increased in the entity, so that the natural habitat of the Burrowing owl has presented a drastic transformation in its use of soil.

During the period from October 2014 to July 2016, 16 transects on road were made in three sites, of 3.4 kilometers in average each one. Every 200 meters were made point-count with call playbacks, of two minutes of duration by one of silence, with three replicas. The observed and heard owls were recorded.

11 individuals of Burrowing Owl were captured with a trained Cooper Hawk (*Accipiter cooperii*), with protectors in the claws to avoid injury to the captured owls. A veterinarian clinically evaluated the health status of the birds, and biometric data (tarsus length and weight) were taken [19], each organism was classified as chick or adult according to

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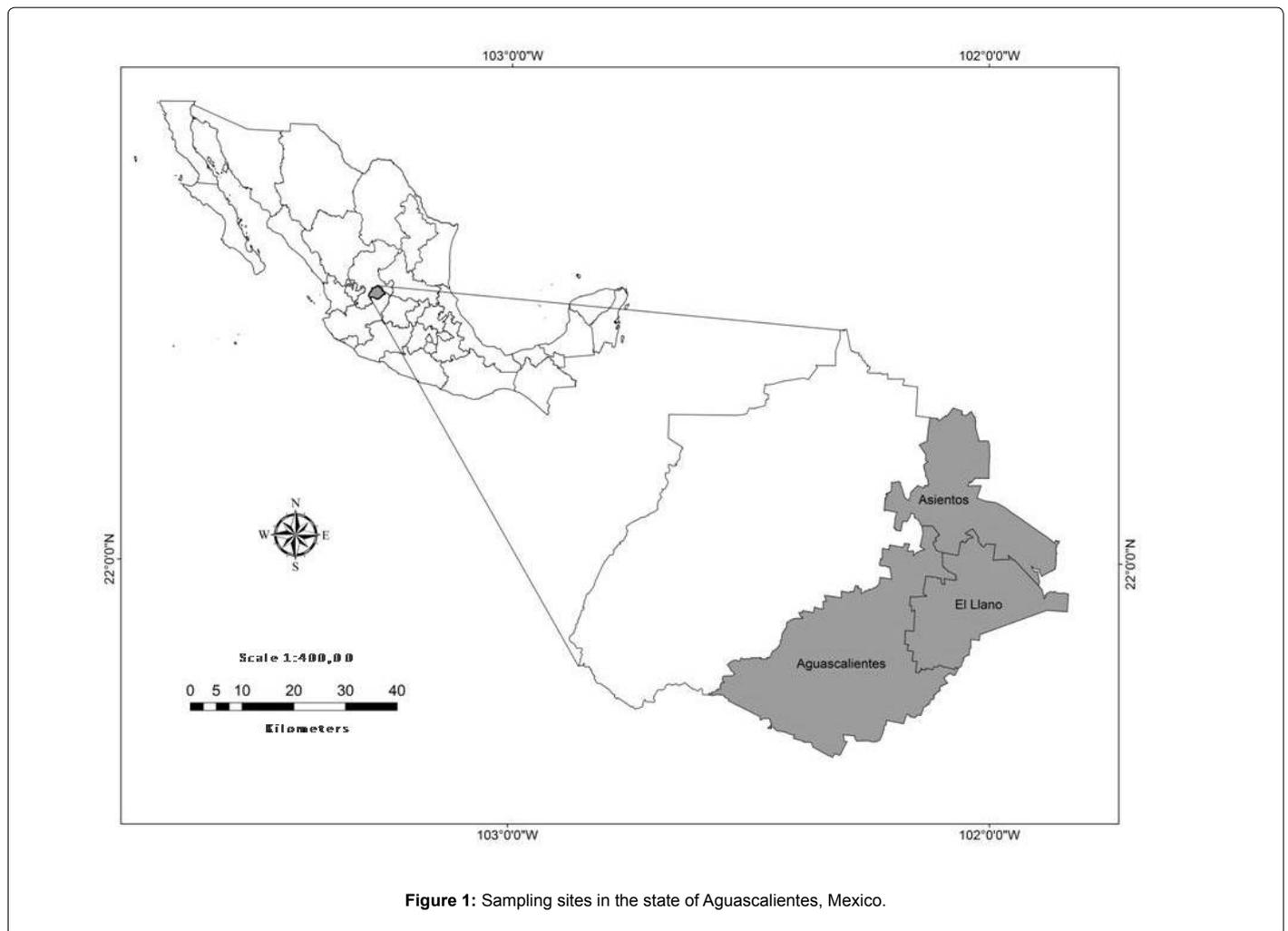


Figure 1: Sampling sites in the state of Aguascalientes, Mexico.

size, weight and plumage. After blood sampling (from the brachial vein, with a 25G needle and hypodermic syringe), the owls were banded as a form of identification and released immediately.

Two blood smears were performed per organism, one for micronucleus test (MN) and other for the leukocyte differential. Both were fixed in ethanol (90%), labeled and stored until were analyzed in the laboratory of International Program of Medicine, of Autonomous University of Guadalajara.

Smears intended for the micronucleus test were processed with the acridine orange technique. The frequency of MNE and NAE was counted in 10,000 total erythrocytes (TE) and the frequency of polychromatic erythrocytes (PCE) in 1000 erythrocytes. Also in 1000 PCE, the frequency of micronucleated PCEs (PCMNE) and PCE with nuclear abnormalities (PCNAE) was counted, trough of microscope equipped under the 100x objective (Zeiss, Axiostar plus model).

For differential leukocyte counts, the smears were stained with the Wright-Giemsa technique, 100 leukocyte cells were counted and the five types were identified: heterophiles, lymphocytes, monocytes, basophils and eosinophils, with the aid of immersion optic microscope (Zeiss, Axiostar plus model). For calculate the heterophil/lymphocyte index (H/L), the number of heterophiles was divided by the number of lymphocytes from each smear [4].

Descriptive statistics (mean, standard deviation and ranges) of the micronucleated cells as well as the frequency of leukocytes, erythrocyte diameters, among others data were calculated. For calculate the abundance of burrowing owl, the average of the monthly records one was divided between the distances of transects in each township. In order to recognize the similarity between the monitored localities, the Bray-Curtis index was applied. The statistical analysis was performed using the software SPSS Statistics 20 (IBM Corp).

Results and Discussion

Owl abundance was low in the most disturbed sites (Asientos 0.15 and Aguascalientes 0.73 birds per km), while the site of El Llano was 2.16 birds per km, a locality in which there are grassland and xerophytic scrub patches (Table 1). The sites Aguascalientes and El Llano showed some degree of similarity (0.49).

It should be noted that the individuals captured, four were chicks which showed higher frequency of PCE (40.8 ± 14.0), MNPCE (22.2 ± 13.8) and NAPCE (6 ± 8.1) than in adults. MNE was similar in chicks (7.8 ± 5.6) and adults (6.6 ± 7.0). However, the frequency of NAE, in adults it was higher (25 ± 15.8) than chicks (Table 2). The high values of EPC in the Burrowing owl chicks are due to the immature reticuloendoplasmic system, which is normal in birds at that age [11] and to constant activity in the production of blood cells, which will decrease until reaching the

homeostasis.

In the differential leukocyte count, lymphocytes were the most abundant in chicks (76.8 ± 10.5), followed by heterophiles (11.3 ± 6.0). In adults the same pattern was observed (lymphocytes 73.2 ± 5.7 and heterophiles 12.2 ± 3.6). With regard to H/L ratio, chicks recorded 0.16 ± 0.09 and adults 0.17 ± 0.06 (Table 2).

High frequency of PCE in adult owls allows inferring, that during the environmental biomonitoring there are genotoxic agents that produce acute effects. The MN test together with H/L index, enable to establish of immunological status and chronic exposure of pollutants; they are respond to different manifestations of the health state of the individuals and reflects the impact about the ecosystem, thus these parameters are a valuable tool in conservation efforts, thus providing early warning of potential damage to environment health, based on wildlife response [20,21].

In raptors from free life, observed that the elevated H/L on individuals exposed to lead [22] and that a poor body condition promotes a high H/L index, suggesting a decrease in immunocompetence or higher stress rates on the birds [23]. The weight and size recorded in the owls

analyzed did not show any relation to the frequency of MN and H/L index, however, it is considered that in diseased birds or pollution, both parameters would be altered. In this study, during the clinical evaluation of the birds, were healthy apparently for showing no signs of disease or injury. Martínez-Quintana et al. [4] suggest that to create the normal baseline parameters, is necessary a sample which preferably represents a broad demographic range of the species, so it is suggested to extend sampling in this owl species and other individuals of the Strigiforme order.

Our result, together with other studies with different species of owls, showed that members of Strigiformes Order are biomonitors acceptable for monitoring genotoxics agents, since species such as the Eastern Screech owl (*Otus asio*), Barn owl (*Tyto alba*) and the owl (*Otus sp.*) recorded acceptable MN frequencies [10,11].

In wild birds from disturbed areas with a persistence of contaminants, there tends to be an increase the frequency of MN in erythrocytes [24]. A similar effect was observed our study area, where in the Aguascalientes site, the frequency of MNE and NAE was higher than the rest of the sampling sites. Currently, this site transformations experiment from anthropic origin that endanger the quality of the habitat. Urban development through constructions for industrial purposes of automobile manufacture and logistic are the main cause, so that natural sites (grasslands and xerophilous scrub) are reduced. While El Llano site consists primarily of grassland patches and agricultural areas, human activity is often restricted to temporary agriculture and livestock farming (intensive and extensive).

It has been suggested to apply the H/L ratio in conjunction with others parameters since by itself is difficult to differentiate between the types of acute and chronic stress [25]. For this reason, it is considered that the combination between the H/L index and the MN test can give an approach to demonstrate the chronic exposure of the etiological agent. Biomonitoring of genotoxic pollutants in fragile habitats such as grasslands provides the guideline for timely corrective actions.

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Biomarkers	Aguascalientes	El Llano	Asientos
Abundance	0.73	2.16	0.15
MNE/10000	10.0	6.7 (6.3)	-
NAE/10000	40.0	18.6 (14.8)	-
MNPCE/1000	0.0	1.7 (2.5)	-
NAPCE/1000	2.0	4.3 (6.4)	-
H/L ratio	0.2	0.2 (0.1)	-

Table 1: Abundance, frequency of de micronucleus and H/L ratio (mean \pm SD) registered in Burrowing Owl at each sampling site.

Parameter	Chicks (n=4)		Adults (n=7)		
	Mean (\pm SD)	Min-Max	Mean (\pm SD)	Min-Max	
Tarsus long (cm)	36.8 (1.4)	35.2- 38.3	52.8 (3.9)	46.1-56.0	
Weight (g)	108.3 (6.7)	100-115	143.4 (16.1)	120-160	
Leukocyte differential /100 leukocytes	Monocytes	9.0 (4.2)	4-14	9.5 (2.7)	6-14
	Eosinophils	2.5 (0.6)	2-3	3.7 (2.2)	1-7
	Basophils	0.5 (0.6)	0-1	1.5 (1.4)	0-4
	Heterophiles	11.3 (6.0)	3-17	12.2 (3.6)	7-17
H/L ratio	0.16 (0.09)	0.03-0.3	0.17 (0.06)	0.09-0.2	
Erythrocytes					
Cytoplasm	Long μ m	14.8 (0.2)	14.6-15.0	14.4 (0.5)	13.7-15.2
	Wide μ m	8.7 (0.6)	8.1-9.2	8.1 (0.4)	7.5-8.6
Nucleus	Long μ m	5.9 (0.1)	5.8-6.0	6.-7 (0.3)	6.3-6.9
	Wide μ m	3.3 (0.1)	3.6-3.4	3.0 (0.2)	2.8-3.5
MNE/10,000 TE	7.8 (5.6)	1-14	6.6 (7.0)	0-20	
NAE/10,000 TE	12.8 (13.2)	2-32	25 (15.8)	10-52	
PCE/1000 TE	40.8 (14.0)	25.1-58.9	22.2 (13.8)	5.8-43.8	
MNPCE/1000 PCE	3 (3-6)	0-8	0.7 (1.1)	0-3	
NAPCE/1000 PCE	6 (8.1)	0-18	3.0 (5.1)	0-14	

H/L- Heterophil/Lymphocyte; MNE- Micronuclei erythrocytes; NAE- Nuclear abnormalities erythrocytes; TE- Total erythrocytes; PCE- Polychromatic erythrocytes; MNPCE- PCE with micronucleus; NAPCE- PCE with nuclear abnormalities.

Table 2: Frequency of erythrocytes with micronucleus and nuclear abnormalities, leukocyte differential and H/L ratio in Burrowing Owl (*Athene cunicularia*).

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