El Colegio de la Frontera Sur

“Cortisol y endoparásitos de mono araña (*Ateles geoffroyi yucatanensis*) bajo diferente grado de presencia humana”

TESIS

Presentada como requisito parcial para optar al grado de

Maestra en Ciencias en Recursos Naturales y Desarrollo Rural

por:

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2014

A toda mi familia, por su apoyo incondicional, y en especial a mi madre y a mi hermana, quienes me han llenado de valor e inspiración a lo largo de éste y muchos otros caminos recorridos; por ser un ejemplo a seguir.

A Orlando, que me motiva y llena de felicidad todos los días, por compartir este difícil camino y muchos otros que faltan por recorrer.

A mis queridos amigos ecosureños con quienes he tenido el placer de compartir risas y llantos en estos años de estudio, en especial a Crisol, Lily, Paola (con H), Jorge y Erika.

Agradecimientos

Al Consejo Nacional de Ciencia y Tecnología (CONACyT) por otorgarme la beca para realizar mi maestría en El Colegio de la Frontera Sur, unidad Campeche. Al Programa de Apoyo a las Tesis de Maestría (PATM) de El Colegio de la Frontera Sur (ECOSUR). Al M. en C. José A. Zúñiga Morales, director de la Reserva de la Biósfera de Calakmul, por otorgarme el permiso para realizar mi investigación en la reserva. A la Dra. Marta C. Romano por permitirme realizar el análisis de cortisol en el Departamento de Fisiología, Biofísica y Neurociencias, Centro de Investigación y Estudios Avanzados del IPN. A Ricardo Valdez, por su asesoría en la realización de la extracción de cortisol y del radioinmunoanálisis.

Al Laboratorio de Parasitología de la FMVZ-UNAM, particularmente al MVZ Julio García Hernández, por su valiosa asesoría en el laboratorio y por su invaluable paciencia y solidaridad.

A mi tutor, el Dr. Rafael Reyna Hurtado y a los Dres. David González Solís, Juan Carlos Serio Silva y Luis Manuel García Feria, por aceptar formar parte de mi comité y brindarme comentarios que han enriquecido el contenido del presente trabajo.

A Alba Rodas y Domingo Ordóñez, por toda su ayuda, dentro y fuera del laboratorio; por guiarme y darme los mejores consejos siempre.

Contenido

I. Introducción

Una meta fundamental en los estudios ecológicos es identificar los factores que influyen en la abundancia de las poblaciones silvestres (Chapman 2006). Uno de estos factores es el surgimiento de enfermedades infecciosas, las cuales tienen un gran impacto particularmente en las poblaciones que se encuentran en peligro de extinción, ya que pueden provocar una disminución poblacional significativa (Altizer *et al.* 2007). Una de las causas del surgimiento y propagación de una enfermedad infecciosa en las poblaciones silvestres es la introducción de nuevos patógenos al hábitat natural de las especies silvestre (Mbora y McPeek 2009; Altizer *et al.* 2007; Karesh 2005) por la interacción -directa e indirecta-, entre el humano y los animales silvestres, o bien, entre sus animales domésticos y los silvestres (Muma *et al*. 2007). A medida que aumenta el grado de intromisión o presencia humana en el hábitat natural, se incrementa el riesgo del surgimiento y propagación de enfermedades infecciosas en las poblaciones silvestres (Gillespie *et al.* 2008; Fiorello *et al.* 2006; Pedersen *et al.* 2005; Patz 2000) y de compartir enfermedades entre humanos, animales domésticos y silvestres (Cook y Karesh 2006; Chapman *et al*. 2005; Suzán 2000). Aunado a esto, se ha demostrado que las poblaciones de primates que habitan sitios perturbados, y de mayor proximidad a asentamientos humanos, presentan mayor prevalencia e intensidad parasitaria que aquellas más aisladas (Mbora y McPeek 2009b; Trejo-Macías 2007; Gillespie y Chapman 2006; Chapman 2005; Daszak 2000). Debido a lo anterior, la riqueza específica, así como la intensidad y prevalencia parasitaria, son considerados indicadores para evaluar el riesgo de adquirir una enfermedad (Nunn y Altizer 2006).

Las enfermedades parasitarias no son frecuentemente letales para el hospedero, pero pueden reducir severamente su función reproductiva e inmunológica (Romero 2004; Toft II Eberhard 1998). A consecuencia de esto, un individuo se vuelve más susceptible al desarrollo de infecciones secundarias y puede afectar la densidad y distribución de la población (Stoner 1996). Los cambios en la prevalencia, intensidad o diversidad parasitaria en una especie hospedera, pueden indicar que ésta ha entrado en contacto con nuevos patógenos, debido a que su hábitat está siendo perturbado (Vitaskova 2007). La introducción de nuevos patógenos o la alteración de la diversidad de los mismos en un ecosistema, puede afectar no sólo la salud de las poblaciones silvestres, sino del ecosistema, al alterar el ensamblaje de los microorganismos dentro de éste (Gómez 2012).

Otra consecuencia de la intromisión humana en el medio natural, es el estrés generado en las poblaciones silvestres. En situaciones de estrés, el organismo aumenta la actividad del eje Hipotálamo-Hipofisiario-Adrenal (HHA), lo que provoca un incremento en la producción de glucocorticoides (cortisol o corticosterona), los cuales pueden ser medidos en suero, orina o materia fecal (Cockrem 2013). Por lo tanto, esto constituye un medio efectivo para evaluar la respuesta fisiológica ante diversos estresores (Cockrem 2013; Romano *et al*. 2010; Brousset 2005; Bahr *et al*. 2000; Whitten *et al*. 1998). A pesar de que la secreción de glucocorticoides es una respuesta que permite la adaptación de los individuos ante un peligro o evento estresante, cuando los individuos los producen de manera constante y prolongada, estos pueden afectar la función reproductiva e inmunológica, por lo que los individuos se vuelven más susceptibles a desarrollar una enfermedad infecciosa. Las altas concentraciones de glucocorticoides pueden producir también alteraciones en el crecimiento, baja tasa de fecundidad, y mayores tasas de mortalidad neonatal (van Heugten *et al*. 2009: Valdespino *et al*. 2007; Nunn y Altizer 2006; Sapolsky *et al*. 2000; Wasser *et al*. 1997).

La determinación de glucocorticoides y de los parámetros endoparasitarios son indicadores que pueden ser evaluados a partir de muestras fecales, por lo que no se requiere de la captura de los individuos bajo estudio (Millspaugh y Washburn 2004), Por esta razón, los niveles de cortisol no se ven alterados y se disminuye el margen de error (Bechert 2012; Hodges 2005; Möstl 2002).

La intromisión humana y la perturbación del hábitat son actividades comunes en muchas Áreas Naturales Protegidas (ANP), debido a las actividades de ecoturismo que se presentan dentro de éstas, así como por los numerosos asentamientos humanos ubicados alrededor de las mismas. En la actualidad, algunas poblaciones de primates no-humanos de la región de Calakmul (Campeche, México), viven en áreas con gran influencia humana, ya que existen alrededor de 100 asentamientos humanos dispersos en bosques ejidales, al exterior de la Reserva de la Biósfera de Calakmul (REBICA). En ellas se llevan a cabo diversas formas de aprovechamiento de los recursos naturales, entre las que destacan la agricultura, ganadería, cacería de subsistencia y algunas de tipo extractivo no maderables, como la apicultura y la obtención de hojas de palmas y semillas (Reyna-Hurtado y Tanner 2010). Por el contrario, dentro de la REBICA, el impacto humano ha ido disminuyendo desde hace al menos 40 años, cuando dejó de haber extracción de chicle en la región y los campamentos antiguos fueron reubicados (Reyna-Hurtado y Tanner 2010). La disminución de este impacto ha dado como resultado la presencia de poblaciones de primates que se mantienen sin contacto alguno con humanos (Reyna-Hurtado com. pers.). Sin embargo, la zona arqueológica, ubicada dentro de la REBICA, tiene una alta y constante afluencia turística, así como una continua actividad de remodelación y construcción.

Dentro y fuera de la REBICA, habitan poblaciones de mono araña peninsular (*A. g. yucatanensis*), una de las tres especies de primates mexicanos. Este primate es clasificado como vulnerable por la Unión Internacional para la Conservación de la Naturaleza (IUCN 2011) y está considerada como especie en peligro de extinción por las leyes mexicanas (SEMARNAT 2010). *Ateles. g. yucatanensis* tiene un papel importante como dispersor de semillas, lo cual favorece la diversidad y resiliencia del ecosistema (González-Zamora 2012). Por esta razón, surge la necesidad de conocer la respuesta fisiológica de esta especie ante la intromisión humana en la región de Calakmul.

Los datos obtenidos pueden aportar información valiosa para la elaboración de estrategias de conservación de ésta y otras especies de primates. Conocer y evaluar dichos indicadores permitirá además detectar situaciones de riesgo epidemiológico tanto para el humano como para la fauna silvestre.

Objetivo general

Determinar si la presencia humana influye sobre las concentraciones de cortisol fecal y los parámetros de infección parasitaria de poblaciones silvestres de mono araña (*Ateles geoffroyi yucatanensis*) en la región de Calakmul, Campeche, México.

Objetivos específicos

1. Determinar los niveles de cortisol fecal de poblaciones silvestres de mono araña que habitan sitios con diferente nivel de presencia humana.

2. Determinar la riqueza específica, prevalencia e intensidad parasitaria de poblaciones silvestres de mono araña que habitan sitios con diferente nivel de presencia humana.

3. Determinar si existe relación entre la concentración de cortisol fecal y los parámetros endoparasitarios de las poblaciones estudiadas.

Hipótesis

- Las concentraciones de cortisol fecal, la prevalencia e intensidad parasitaria serán mayores en las poblaciones de mono araña que habitan un sitio con alto nivel de presencia humana, que en uno con baja presencia.
- Existirá una relación positiva entre los parámetros de infección parasitarios y los niveles de cortisol fecal de las poblaciones de mono araña.

II. Gastrointestinal parasites and fecal cortisol of spider monkey (*Ateles geoffroyi yucatanensis*) in locations with different level of human presence*

*Manuscrito enviado a la revista *International Journal of Primatology*

Gastrointestinal parasites and fecal cortisol of spider monkey (*Ateles geoffroyi yucatanensis*) in locations with different level of human presence

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Abstract Infectious diseases have a major impact on wildlife populations, particularly those that are in danger of extinction, such as the peninsular spider monkey (*Ateles geoffroyi yucatanensis*). Habitat disturbance and the increased intrusion of humans and their domestic animals into its habitat, make possible the introduction of novel pathogens and the establishment of a prolonged response to stress, causing physiological alterations that affect the overall health status of individuals from wild populations. The present study analyzed the effect of human presence on fecal cortisol concentration, and in the prevalence and intensity of parasite infection in wild populations of *A. g. yucatanensis* inhabiting wild sites with different level of human disturbance in the Calakmul Region, Campeche, México. One hundred and twenty fecal samples collected in dry season were analyzed to detect parasites by flotation technique and fecal cortisol by radioimmunoassay. Our results suggest that human presence is affecting the overall health status of wild primate populations by causing an increase in cortisol levels as well as affecting host-parasite dynamics, which increases the disease risk and vulnerability of this endangered species.

Key words *Ateles*, glucocorticoids, parasites, stress response, Calakmul

Introduction

Recent studies have suggested that infectious diseases have an important impact on wildlife populations, with a marked effect on endangered species because of a significant population loss on an already reduced population (Altizer *et al*. 2007). One cause of the emergence and spread of a disease in a wild population is the introduction of novel pathogens into an habitat (Mbora and McPeek 2009; Altizer *et al.* 2007; Karesh 2005) due to an interaction –directly and indirectly- between humans, domesticated animals and wildlife (Muma *et al.* 2007). As human intrusion into wild population´s habitat increases, the risk of emergence and sharing infectious diseases among humans, domestic animals and wildlife, increases as well (Gillespie *et al.* 2008; Fiorello *et al*. 2006; Cook and Karesh 2006; Chapman *et al*. 2005; Suzán 2000).

Existing research has shown that anthropogenic factors can affect host-parasite dynamics in free-ranging primate populations (Mbora and McPeek 2009b; Trejo-Macías 2007; Gillespie and Chapman 2006; Chapman 2005; Daszak 2000), causing an increased parasite prevalence and parasite intensity in those primate populations inhabiting perturbed sites or in proximity with human settlements. Therefore, parasite richness, prevalence and intensity, are considered indicators of disease risk (Nunn and Altizer 2006). Likewise, it has been observed that parasitic infections, even when mild, can cause major physiological alterations to the individual, affecting its reproductive capacity and compromising their immune system (Wobeser 2013; Romero 2004; Toft II and Eberhard 1998). As a result of this, a parasitized individual becomes susceptible to the development of secondary infections, which has relevant epidemiological implications and large impact within a population by the rapid spread and high rates of mortality caused by some infectious agents. Changes in parasitic prevalence, intensity or diversity in a host species, may indicate that this last one has come in contact with new pathogens or that their habitat is being disturbed (Gómez 2012; Vitaskova 2007).

Another consequence of the human interference is the stress generated in wild populations. Stress is a general syndrome of adaptation to a stimulus (stressor), either the shortage of food (Chapman *et al*. 2006), social interactions, fear, pain (Wobeser 2006), presence of predators or humans (Rangel-Negrín 2009; Davis 2005), etc. In a stressing situations, individuals respond to the stressor by activation of the Hypothalamus- Pituitary-Adrenal (HPA) axis, that results in the increased secretion of glucocorticoids (cortisol or corticosterone) (Cockrem 2013) In spite of the fact that the secretion of glucocorticoids is a response that allows adaptation of individuals to a danger or stressor, when individuals are exposed in a constant and prolonged manner to a stressor, glucocorticoids can affect the immune system and reproductive function, so that this individual becomes more susceptible to develop an infectious disease. High concentrations of glucocorticoids can also produce alterations in growth; low fertility rates and higher rates of neonatal mortality (van Heugten *et al*. 2009; Valdespino *et al*. 2007; Nun and Altizer 2006; Sapolsky *et al*. 2000; Wasser *et al*. 1997). Determination of glucocorticoids and parasitic parameters are health indicators (Brousset 2005) that can be evaluated from fecal samples, and do not require the capture of the individuals under study (Millspaugh and Washburn 2004), which is why the levels of cortisol are not altered, thus decreasing the margin of error in hormone determinations (Bechert 2012; Hodges 2005; Möstl 2002); therefore, they can be an effective way to assess wildlife health and stress response to human perturbation (Cockrem 2013; Romano *et al*. 2010; Bahr *et al*. 2000; Whitten *et al*. 1998).

Human presence and habitat perturbation are common activities outside Natural Protected Areas (ANP by its Spanish initials), but due to ecotourism, research and other non-extractive activities inside of them, human perturbation is becoming common inside ANP. In addition, there are numerous human settlements located around many ANPs. Currently, about 100 human settlements are scattered outside of the Calakmul Biosphere Reserve (REBICA), in Campeche, México. For this reason, some populations of non-human primates in Calakmul region live under human influence. Human activities outside the REBICA occur in the many forms of exploitation of natural resources, among which are agriculture, livestock and subsistence hunting (Reyna-Hurtado and Tanner 2010). These external areas are community forests (ejidos) where in addition to the previously identified activities there are some type of non-timber extraction as apiculture, collecting of palm leaves and seeds, among others. In the other hand, inside the REBICA, human impact has been declining since at least the last 40 years, when chewing gum extraction stopped in the region and the old camping sites were relocated. The reduction of this impact results in the existence of some primate populations maintained without any contact with humans (Reyna-Hurtado pers. comm.). However, the archaeological area of Calakmul, located within the REBICA, has a high and constant influx of tourists, as well as a continuous activity of remodeling and construction which put in contact humans and primates in this area inside the ANP.

Currently, inside and outside of the REBICA inhabits populations of peninsular spider monkey (*A. g. yucatanensis*), one of the three species of Mexican primates. This primate is classified as vulnerable by the International Union for the Conservation of Nature (IUCN 2014); and under Mexican law is considered as an endangered species (SEMARNAT 2010). *A. g yucatanensis* has an important role as seed disperser, which favors the biological diversity and forest resilience processes (González-Zamora 2012). For these reasons, there is a need to know the response of this species to human pressure in the Calakmul region. To achieve the above, the aim of this study was to determine fecal cortisol concentrations and investigate endoparasitic parameters (parasite richness, prevalence and intensity) in wild populations of spider monkey (*A. g yucatanensis*) that inhabit sites exposed to different level of human presence, in Calakmul Region. The derived data can provide valuable information for the development of conservation strategies for this and other primate or wildlife species. Assessment of these indicators can also allow detection of epidemiological risk for both human and wildlife populations.

Methods

Study area

The current study was carried out in the Region of Calakmul, in two areas of continuous forest, one of them located within the REBICA and the other one located outside, in the Ejido Nuevo Becal, both located in the Municipality of Calakmul, Campeche, Mexico. The REBICA represents the most important region of tropical forest of Mexico and has a surface of protected area of 7238 km^2 in which predominate semi-evergreen forest (Galindo-Leal 1999). Nuevo Becal is a community forest located in the northeast of the REBICA. It possesses a great extension of continuous forest (520 km^2), where daily extractive and non-extractive activities such as agriculture, selective logging, beekeeping, chewing gum extraction from zapote tree, palm-leaves and seed collection, sport and subsistence hunting, are carried out (Reyna-Tanner 2010). Calakmul forest is part of the Mesoamerican Biological Corridor, where there is a flow of species from the Yucatecan biotic province, of the Isthmus of Tehuantepec and the Guatemalan Peten. The surface is fairly homogeneous and has an altitude that ranges from 260 to 385 meters above sea level. The climate is warm humid, with an average annual high temperature of 27° C and a low of 18 °C, with an annual precipitation range of 600-1200 mm (Galindo-Leal 1999).

Sampling sites

We sampled groups of *A. g. yucatanensis* inhabiting sites with different level of human presence. Six sleeping sites (Fig. 1) were located and categorized as *low, medium* and *high* level of human presence, as follow:

a) Inside the REBICA:

1. Low Level: *Barranco Reyna* (18° 14'11.4" N, 89° 51 '35.2" W). Continuous and conserved forest isolated from tourists and staff of the REBICA. Sporadic presence of researchers.

2. Medium Level: *El Ramonal* (18° 18' 59.5" N, 89° 51' 28.8" W). Continuous and conserved forest located at kilometer 27 of the road that leads to the archaeological area. Sporadically visited by tourist and researchers.

3. High Level: Archaeological site (18° 06' 34.2" N, 89° 48' 17.5" W). Continuous and conserved forest located at kilometer 60 inside the REBICA. Surrounded by highly conserved forest. Daily visited by tourists, with a mean of 27500 tourists annually (Coutiño-CONANP 2014, pers. comm.).

b) Outside of the REBICA (Nuevo Becal):

4. Low Level: *Área semillera* (18° 45' 40.9" N, 89° 12'14.2" W). Highly conserved forest, located 14 km away from human settlement (Nuevo Becal). Due to its remoteness, it is rarely visited by people from Nuevo Becal.

5. Medium Level: *El Naranjalito* (18° 35' 46" N, 89° 11' 14" W). Highly conserved forest area located 8 km away from Nuevo Becal. Some people have crop lands and cattle production there. This site is almost daily visited by some farmers, who get there by motorbike or horseback riding.

6. High Level: *El Porvenir* (18° 33' 28.6" N, 89° 18' 01.7" W). Highly conserved forest area located 4 km away from Nuevo Becal. Daily visited mainly by men and young people from town on a motorbike or horse, for extractive and recreation activities.

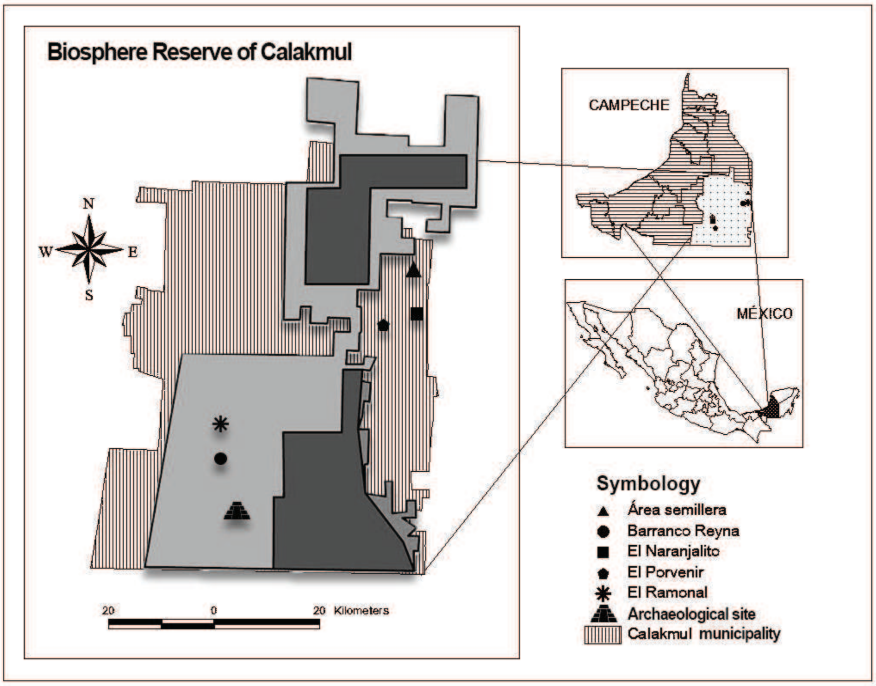


Fig. 1 Location of sites where fecal samples of *A. g. yucatanensis* were collected

Fecal Sampling and preservations methods

Between February and May 2013, the core of the dry season, we collected 120 fecal samples of *A. g. yucatanensis*. All samples were fresh and collected from the first defecation in the morning, at around 6 a.m. in an attempt to avoid diurnal variation in the cortisol production (Behie *et al.* 2010).

Sampling was carried out in a different site each day so the stress caused by our presence while sampling, was not reflected in the fecal cortisol concentrations. Fecal samples were transported in a portable ice pack to the camp, where each sample was divided in two equal parts, one of theme was preserved in 4% formalin, for parasitological evaluation; the other remained in the zip-lock bag and was stored at -20 ^{0}C until air transportation to México City, where samples were subsequently stored at -24 ^{0}C in the Department of Physiology at the Center for Research and Advanced Studies (CINVESTAV by its Spanish initials).

Parasitological assessment

Parasite identification was conducted in the Laboratory of Parasitological Diagnosis at the Veterinary Medicine School, in the National Autonomous University of Mexico (UNAM). Flotation was used for parasite species identification and Kinyoun staining technique (Foreyt 2001) was used to confirm the presence of *Cryptosporidium* sp. Eggs and oocyst identification was based on its size, shape, color and content, in an optical microscope. Each slide was systematically observed under 10x and 40x objectives. A modified McMaster technique (Foreyt 2001) was used for egg/oocysts counting.

Parasitic infection was described in terms of prevalence (proportion of infected individuals), richness (number of species of parasites observed) and parasite intensity (number of eggs/oocysts per gram of feces). Because it was not possible to carry out the individual recognition of sampled animals, it is feasible that some of them were sampled more than once, therefore prevalence should be interpreted as an index of parasite infection. However, probability of collecting a sample from the same individual is minimal because we switch places every other day and there is some evidence that sleeping sites are not visited by the same individuals every night. In addition, the probability of having repeated samples is the same in the six sampling sites, so results should not be affected (Chapman, pers. comm.).

Cortisol extraction and radioimmunoassay (RIA)

In the lab, once plant compounds (seeds, leaves) were removed, each sample was dried in an oven (Precision MR) at 62 °C ± 3, until complete dryness was reached. Dried samples were pulverized and 0.4 g was weighed and placed in sterile 16 x 125 mm tubes. Five milliliters of ethanol (80 %) were added to each tube, and vortexed for ten minutes. Tubes were then placed on a water bath at 80 ^{0}C for 20 min. Ethanol was added to compensate for the evaporated solvent and tubes were centrifuged at 2500 rpm for 20 minutes, then the supernatant was decanted into new tubes to be dried with pressurized air while keeping the tubes in a water bath at 36° C. Three hundred µl of ethanol were added to each tube, that were mixed in a vortex for 10 minutes. After 30 min of incubation at room temperature, samples were centrifuged again for 20 min at 2500 rpm. Finally, the supernatant was decanted into tubes and stored at -24 °C until used for RIA.

Radioimmunoassay was carried out by the method of Parrot and collaborators (1989), using a CORT-CT2 Kit (Cisbio Bioassays, France, 2013), with rabbit anti-cortisol. The antibody cross-reactions were 2.5 % for corticosterone, 0.01 per cent for progesterone, <0.01 % for testosterone and 0.02 % for aldosterone. Iodine (^{125}I) was used as the tracer.

Food Availability

To reduce the bias error of the possible effect of food availability on fecal cortisol concentrations and the parasitic parameters, we also evaluated this variable.

Determination of food availability was based on the measurement of the diameter at breast height (DBH) of trees that were counted in a designed net of transects (Stevenson 2001; Chapman 1992). Systematic sampling was carried out by establishing a transect of one kilometer long by 10 meters wide, in each of the six sampling sites by a total of six transects one kilometer each. All trees of those species that are commonly consumed by the spider monkey, according to the literature (Sarabia-Hernández unpublished; Di Fiore 2008) and own observations, and that had a DBH greater than 20 cm were registered. For each site the total DBH was calculated. The identification of tree species was done in field with the collaboration of four local guides with extensive knowledge of local vegetation.

Statistical analysis

Non-parametric tests were used because the data obtained did not have normal distribution. Kruskal-Wallis was used to analyze differences in cortisol levels between the three groups within and outside the reserve, while Mann-Whitney test was used to analyze differences between the values of cortisol and the parasite intensity obtained within the reserve, with those obtained outside of this. Using linear regression analyzes we defined the relationship between the level of human presence and the concentration of cortisol, the DBH and the parasite intensity. Statistical analyzes were performed with GraphPad PrismV5 for Windows (GraphPad Software, Inc., La Jolla, CA) and IBM SPSS Statistics 19.

Results

Parasitological assessment

We analyzed 120 fecal samples from *A. g. yucatanensis*. Sixty six of those samples were obtained within the REBICA (N=26 Archaeological site; N=20 El Ramonal; N=20 Barranco Reyna) and 54 samples outside of this, at Nuevo Becal (N=22 El Porvenir; N=14 Naranjalito and N=18 Área semillera). Forty three out of 120 samples (35.83%) were positive for at least one parasite species. We identified three different endoparasites (two of them were only identified as genera and one to the level of species): *Cryptosporidium* sp. (coccidio), *Controrchis* sp.(trematode) and *Trypanoxiurys* sp. (nematode).

The highest prevalence of infection was 61.5 %, in the groups of *A. g. yucatanensis* inhabiting the REBICA archeological site. El Ramonal and Barranco Reyna, showed a lower prevalence (30 % y 35 %, respectively). At Nuevo Becal, prevalence of infection was 44.4 %, 21.4 % and 13.6 % for Área semillera, Naranjalito and El Porvenir, respectively.

Within REBICA, *Trypanoxyuris* sp. had a prevalence of infection of 30 %, 20 % and 50 %, at Barranco Reyna, El Ramonal and the Archeological site, respectively, while at Nuevo Becal prevalence of *Trypanoxyuris* sp. was 38.9 %, 21.4 % and 13.6 %, at Área semillera, Naranjalito and El Porvenir, respectively. In the case of *Cryptosporidium* sp., prevalence of infection was 5 %, 10 % and 30.8 %, for groups inhabiting Barranco Reyna, El Ramonal and the Archeological site, respectively. In Nuevo Becal, *Cryptosporidium* sp. was only observed at El Porvenir, with a prevalence of 4.5 %. *Controrchis* sp.*,* was only observed in samples collected at Área semillera with a prevalence of 5.5 % (Table I).

Table I Prevalence (percent of individuals) of parasites identified in fecal samples of *A. g. yucatanensis* collected in locations with different level of human presence.

Sampling site	Level of Human presence	Total Prevalence	*Trypanoxyuris* sp.	*Criptosporidium* sp.	*Controrchis* sp.	>1 spp.
REBICA						
Barranco Reyna (N=20)	Low	35	30	5	0	0
El Ramonal (N=20)	Medium	30	20	10	0	5
Archaeological site (N=26)	High	61.5	50	30.8	0	19.2
Nuevo Becal						
Área semillera (N=18)	Low	44.4	38.9	0	5.5	0
Naranjalito (N=14)	Medium	21.4	21.4	0	0	0
El Porvenir (N=22)	High	13.6	13.6	4.5	0	4.5

Multiple infection (presence of more than one parasite species) was higher at the Archeological site, with 19.23 % of the samples presenting *Trypanoxiurys* sp. and *Cryptosporidium* sp. The greatest parasite intensity for *Cryptosporidium* sp. and *Trypanoxyuris* sp. was observed in the Archeological site and El Porvenir (Table II). DBH showed no significant effect on parasite intensity (p=0.49) but in contrast, human presence did show to influence significantly (p=0.013) this variable in all the sites included in this study.

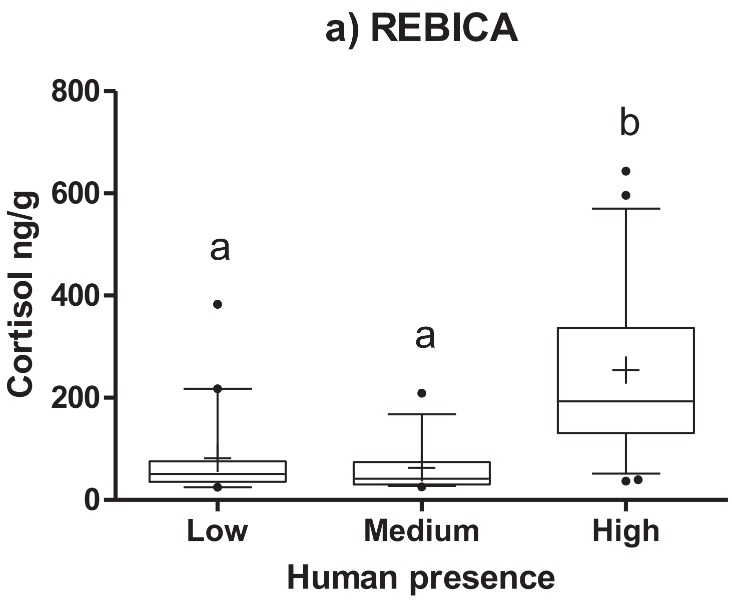
Table II Parasite intensity (eggs/oocysts per gram of feces)

Sampling site	*Cryptosporidium* sp.	*Trypanoxyuris* sp.	*Controrchis* sp.
REBICA			
Barranco Reyna	2450	566.6	0
El Ramonal	101100	387.5	0
Archeological site	104422.2	2038.5	0
Nuevo Becal			
Área semillera	0	618.7	50
El Naranjalito	0	133.3	0
El Porvenir	250	633.3	0

Cortisol assessment by Radioimmunoassay (RIA)

Five of the 120 samples were not enough to carry out both analysis (parasite assessment and radioimmunoassay), therefore only 115 samples were analyzed by RIA. Fecal cortisol values for *A. g. yucatanensis* from REBICA (n=64), ranged from 24.9 ng/g to 643.8 ng/g, while in Nuevo Becal (n=51) cortisol values ranged from 27.89 ng/g to 483 ng/g. No significant differences were found between values from both sites (Kruskal-Wallis Test, p=0.099). However when Dunn's multiple comparison tests were used to assess differences between the three levels of human presence in REBICA (Low, Medium and High), and highly significant differences in cortisol values were found between sites with low and medium level compared with those sites of high level of human presence (Fig.2a). In this site, the highest mean cortisol concentration (254.4 ± 177.6 ng/g) and the maximum value (643.8 ng/g) were obtained at the high level site (Archeological site), while at El Ramonal and Barranco Reyna, lower concentrations of this hormone were obtained (62.75 ± 50.92 ng/g and 81.79 ± 88.21 ng/g, respectively).

At Nuevo Becal, located outside REBICA, no significant differences in the mean cortisol concentrations were observed among levels of human presence (p=0.06). However, the highest mean cortisol concentration was obtained at the site with high level of human presence (El Porvenir, 170.3 ±131 ng/g) and the lowest mean cortisol concentration at the more isolated site (Área semillera, 126.5 ± 71.4 ng/g) (Fig. 2b). For all sites, parasite intensity showed no significant effect on cortisol concentration (p=0.39) while human presence (p = 0.02) and DAP (p=0.004) showed a strong influence on the values of this hormone.



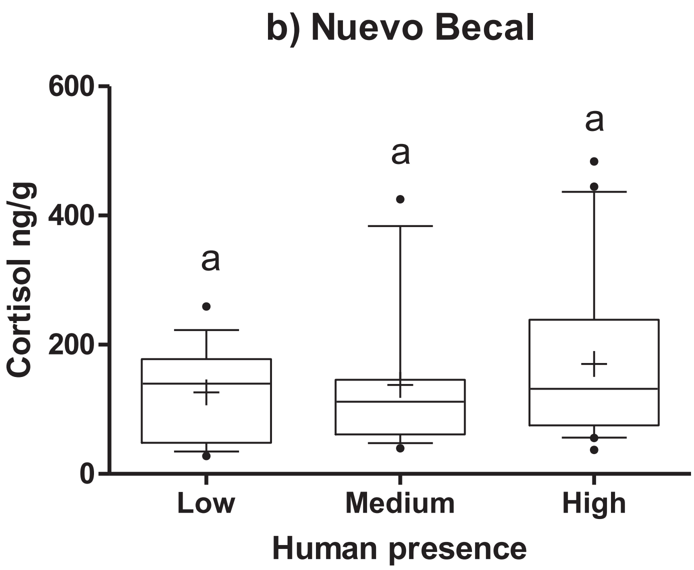


Fig 2. Fecal cortisol concentration in *A. g. yucatanensis* across levels of human presence, in REBICA (a) and Nuevo Becal (b)

Food availability

No statistical significant difference in DBH and arboreal richness among sampling sites (X^2= 5, df=1, P > 0.05) were found, but there was a trend toward greater abundance at Área semillera (527 trees), while the lowest was observed at El Ramonal (127 trees). The highest species richness of arboreal species commonly ingested by spider monkeys were found at the Archeological site where the highest DBH was detected (Table III) although again no significant differences were found.

Table III Tree abundance (number of individuals), DBH (cm) and tree-species richness (number of species) among sampling sites

Sampling site	Tree abundance	DBH	Species Richness
REBICA			
Archeological site	437	32211	17
Barranco Reyna	329	18281	13
El Ramonal	127	10072	14
Nuevo Becal			
El Porvenir	414	24157	17
El Naranjalito	378	25719	15
Área semillera	527	29306	14

Discussion

The present study reports for the first time fecal cortisol concentrations as well as parasite parameters of wild populations of *A. g. yucatanensis*, an endangered species, in the State of Campeche.

Our results are congruent with previous observations in wild populations of peninsular spider monkey (*A. g. yucatanensis*) in Quintana Roo, Mexico (Rangel-Negrín 2009) and those carried out in captive conditions (Davis 2005), in which a positive correlation between human presence and cortisol levels was observed. Likewise, it has been observed that populations of howler monkey (*Alouatta pigra*) inhabiting sites with exposure to tourists have higher levels of cortisol than those who live in places where there is none (Behie *et al*. 2010). These observations are supported by ethological observations (Chamove 1988) where captive primates in a zoo increase 600 % their aggressive behaviors and 300 % their locomotion activity when they are faced with a higher density of spectators and when these show harassment behaviors toward theme.

In spite of the lack of significant differences in food availability among sampling sites, statistical analysis showed that DBH had a negative correlation with cortisol concentration, which is reasonable since DBH is associated with the amount of fruit that a tree produces (Chapman 1994). Thus, at sites with low DBH there is a lower fruit availability, so that individuals do a greater foraging effort, which causes an increase in the activity of the HHA axis and therefore in the production of cortisol (Valdespino 2010; Martínez-Mota 2007; Chapman 2005). However, within REBICA we did not found this relationship since at the Archaeological site, we obtained the highest values and the highest mean concentration of fecal cortisol in spite of having the greatest DBH and arboreal richness. These results suggest that other causes could be responsible of the enhanced cortisol concentrations found in the monkeys living in those sites. On the other hand, we obtained the lowest mean cortisol levels at El Ramonal, in spite of presenting the lowest DBH, which suggest that DBH is not a critical factor in the stress response of this species in Calakmul region.

As well as the fecal cortisol concentration, parasitic parameters in non-human primates can be affected by habitat fragmentation (Bonilla-Moheno 2002; Trejo- Macías 2007) and proximity to human settlements (Ekanayake *et al*. 2006; Vitazkova 2006). Our study showed that in REBICA, the prevalence of infection, parasitic intensity and incidence of multiple infections was significantly higher in individuals inhabiting sites with high level of human presence, which could be of concern since multiple infection has been associated with higher morbidity and mortality in primates (Gillespie *et al*. 2005). This pattern was not observed at Nuevo Becal, in fact, individuals from Área semillera (location of low level of human presence), showed higher prevalence of infection but lower concentration of fecal cortisol than individuals from the other two sites (medium and high level of human presence); as intensity and prevalence may increase with several factors that were not evaluated in the present study, further studies would be needed to define the cause of this result. Nevertheless, the repeated use of sleeping sites or specific deposition sites may increase parasite infection rates (Nunn and Heymann 2005). The later may be a fact influencing our results since individuals from Área semillera were always found at the exactly same place before and after sampling, thus using their sleeping site as latrine and foraging place, in contrast with the other five sampled groups which used different spots of the sampling site and always left the place after defecation. This also could mean that individuals at Área semillera spend more time at the same place, which may increase contact rate and therefore the probability of parasite infection (Nunn and Altizer 2006). The lower cortisol levels observed in this group may be due to the fact that Área semillera has a greater DBH, thus individuals have lower foraging effort and lower nutritional stress (Chapman *et al.* 2006), besides the fact that this group leaves far away from human settlements, therefore exposition to humans and novel pathogens decreases.

The endoparasite species identified in this study have previously been reported in *A. g. yucatanensis* in Quintana Roo, Mexico (Bonilla-Moheno 2002). *Trypanoxiurys* sp. and *Controrchis biliophilus*, have also been reported in *A. geoffroyi* in Costa Rica (Maldonado *et al*. 2014) and mantled howler monkey (*Alouatta palliata*) in the Mexican southeast (Trejo-Macías 2007). Prevalence of *Trypanoxiuris* sp. observed in our study is higher than those previously reported in Quintana Roo (7.69 %, Bonilla-Moheno 2002) but prevalence of *Cryptosporidium* sp. was lower than the one presented in that study (77 %, Bonilla-Moheno 2002), where parasite prevalence and intensity showed a positive correlation with level of human presence. This pattern is consistent with the premise that in habitats fragmented by humans, primate populations are subjected to greater contamination from humans and domestic animals, than populations in continuous habitats (Chapman *et al.* 2005). This finding is also congruent with previous observations (Ekanayake *et al*. 2006), where a higher incidence of *Cryptosporidium* sp. is reported for non-human primates inhabiting sites that present water contaminated with human faeces. Cryptosporidiosis has been reported as an emerging disease in the tropics and causes diarrheic symptoms in humans (Foreyt 2005). The sporulated oocysts are excreted by the infected host through the stool and transmission occurs primarily by consumption of contaminated water.

Environmental contamination with human excreta is recognized as a potential mean of transmission of pathogens, such as *Giardia* sp. (Vitazkova 2006) and *Cryptosporidium* sp. (Appelbee, Thompson and Olson 2005). The fact that we find a higher prevalence and intensity of this parasite within REBICA, suggests a mishandling of the latrines of the archeological site and could become an epidemiological concern for both human and non-human primate populations. Nevertheless, to confirm this supposition fecal and water epidemiological surveys should be done to confirm the presence of *Cryptosporidium* in REBICA´s employees and inhabitants of Nuevo Becal.

Multi-host parasites have been implicated in the emergence of new diseases in humans and wildlife, yet little is known about factors that influence the host range of parasites in natural populations (Pedersen *et al*. 2005). *A. geoffroyi* has an arboreal lifestyle (Campbell *et al.* 2005), with a limited contact with the ground, however there are reports of sightings of this species drinking directly on bodies of water in the soil (Campbell *et al.* 2005) or travelling short paths in the ground occasionally (Reyna-Hurtado, pers. obs). In addition, existing evidence show that *A. geoffroyi* and sympatric species, such as black howler monkey (*A. pigra*) (Bonilla-Moheno 2002) and mantled howler monkey (*A. palliata*) (Maldonado *et al.* 2014), can share even 100% of their infectious agents. The foregoing may be due to their ecological similarity and the fact that they live in sympatry, more than the phylogenetic proximity (Nun and Altizer 2006). The importance of this lies in the fact that sympatric species can share a large number of infectious pathogens, thus increasing disease risk (Davies and Pedersen 2008).

Parasites present in a host act as commensals most of the time, however they may increase its pathogenicity when the individual is immunologically or nutritionally depressed, or when there is an unprecedented increase in the abundance of the parasitic agent (Nunn and Altizer 2006; Chapman *et al*. 2006; Martínez-Mota *et al*. 2007). The latest suggests that those populations of *A. g. yucatanensis* presenting high concentrations of cortisol, high parasitic intensity and low food availability, are more likely to develop ~~an~~ infectious diseases than individuals that have low concentrations of cortisol, low parasite intensity and inhabiting sites with high food availability. In the archaeological site, despite individual presented the highest concentrations of fecal cortisol and the highest prevalence of infection and parasite intensity, a greater food availability was observed, which may be helping to decrease the disease risk. Food availability, parasitism and stress, may have a synergistic effect on the abundance of primates (Chapman *et al*. 2006). For this reason, monitoring fecal cortisol and parasitic parameters must be part of the strategies for the conservation of non-human primates, in addition to the protection of the natural areas.

The results obtained in this study indicate that the increased human presence in the archeological site of Calakmul may have important long-term implications for the populations of *A. g yucatanensis* inhabiting the place. The increased pollution due to the high influx of tourists into the site represents a challenging goal in primate conservation. Measures should be implemented to avoid soil and water contamination with microbes and parasites because this situation combined with human population growth in the surroundings and the increasing human intrusion into natural areas may endanger the spider monkey survival.

Despite the results showed in the present study are limited to evaluate the overall health status at a population level, they provide valuable information that enables better understanding of the factors influencing the vulnerability of spider monkey populations to develop an infectious disease (Nunn and Altizer 2006), as well as the host-parasite dynamic.

Acknowledgments

This study was supported by scholarship (423725/269900) granted by CONACYT-México. We thank all the help offered by the Department of Physiology-CINVESTAV-IPN and the Parasitological Diagnosis Laboratory-FMVZ-UNAM, especially MVZ Julio García Hernández. We thank the authorities of Biosphere Reserve of Calakmul and Nuevo Becal, as well as the collaboration of our field guides, especially Maynor Hernández.

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III. Conclusiones

El análisis estadístico revela que existe una relación positiva entre el nivel de presencia humana y los valores cortisol fecal e intensidad parasitaria de los individuos de *A. g. yucatanensis* muestreados dentro de la REBICA. Sin embargo, el patrón observado en los valores de prevalencia e intensidad parasitaria de los individuos muestreados fuera de la REBICA, no fueron los esperados. Así mismo, los valores de cortisol obtenidos en dichos individuos no mostraron diferencias significativas en relación al nivel de presencia humana, a pesar de que estos valores mostraron un comportamiento ascendente en relación al nivel de presencia humana, obteniendo la mayor concentración promedio de cortisol en el sitio de alto nivel de presencia humana. Lo anterior, en conjunto con los resultados obtenidos en los sitios de muestreo localizados dentro de la REBICA, particularmente los de la zona arqueológica, sugieren un efecto desfavorable de la presencia humana y la perturbación del hábitat sobre las variables analizadas.

A diferencia de lo esperado, no se encontró una relación positiva entre la concentración de cortisol fecal y la intensidad parasitaria en ninguno de los sitios de muestreo. Por otra parte, al contrario de lo esperado y de lo observado dentro de la REBICA, fuera de ésta la mayor prevalencia parasitaria se observó en el sitio de menor nivel de presencia humana, lo cual concuerda con observaciones previas, en donde los sitios menos perturbados por humanos son los que presentan la mayor prevalencia de infección parasitaria probablemente debido a que en estos sitios no se ha roto la red ecológica que involucra la transmisión de agentes infecciosos (Stuart 1993, Bonilla-Moheno 2002).

Las muestras positivas a parásitos no demuestran si la presencia de estos parásitos está provocando enfermedad o simplemente se trata de una infección subclínica. Por otro lado, las muestras negativas a parásitos no demuestran que el parásito no esté presente en el individuo muestreado, debido a que la excreción de parásitos es intermitente (Bowman y Georgi 2009). Sin embargo, esta probabilidad es la misma en los seis sitios de muestreo, por lo tanto no afecta la veracidad de los resultados.

La relación positiva entre el nivel de presencia humana y prevalencia e intensidad de *Cryptosporidium* sp. observada en las poblaciones de mono araña, puede representar un riesgo de transmisión bidireccional entre humanos y primates no-humanos, y constituir un problema de salud pública y una amenaza para las poblaciones de *A. g. yucatanensis* y otras especies de la región de Calakmul. Sin embargo es necesario realizar un monitoreo parasitológico que incluya a la población humana que trabajar en la REBICA así como a los habitantes de Nuevo Becal para evaluar dicho riesgo.

El mono araña tiene un estilo de vida arbóreo, lo cual limita su contacto con el suelo y por ende la adquisición de agentes patógenos de ciclo directo, sin embargo, la perturbación del hábitat pueden generar cambios etológicos, provocando que esta especie se ve en la necesidad de bajar al suelo y entrar en contacto con diversos patógenos. Aunado a esto, las evidencia existente de parásitos que comparten *A. g. yucatanensis* y otras especies simpátrica (Maldonado *et al.* 2014, Bonilla-Moheno, 2002), predispone a al mono araña a adquirir nuevos agentes infecciosos, dando pie a la emergencia de enfermedades dentro de la población.

La disponibilidad de alimento mostró ser un factor que influye sobre la producción de cortisol, sin embargo los individuos de *A. g. yucatanensis* muestreados en la zona arqueológica, presentaron las mayores concentraciones de cortisol fecal a pesar de que estos habitan en el sitio con el mayor DAP registrado. Esto sugiere que incluso en sitios con alta disponibilidad de alimento, la presencia humana puede provocar en el individuo una elevada producción de glucocorticoides y una mayor intensidad parasitaria.

A pesar de que la secreción de cortisol (y otros glucocorticoides) es una respuesta inmediata que permite la adaptación y supervivencia de los individuos ante un peligro o agente estresante, las elevadas concentraciones de esta hormona por un periodo prolongado (estrés crónico) tienen efectos perjudiciales en la función reproductiva e inmunológica de los individuos, lo que compromete la viabilidad de las poblaciones.

El monitoreo de cortisol fecal y los parámetros de infección parasitaria, representa una herramienta no invasiva, de gran utilidad en el monitoreo de las poblaciones silvestres de primates no-humanos, la cual puede generar información relevante para detectar cambios en la salud a nivel individual, poblacional y del ecosistémico. El análisis parasitológico permite, además, la identificación de cambios en la dinámica parásito-hospedero, y aporta información para crear estrategias de conservación oportunas y efectivas.

El presente estudio ofrece los primeros datos sobre cortisol fecal y endoparásitos de mono araña (*A. g. yucatanensis*) en el estado de Campeche, mismos que podrán servir para realizar futuros estudios comparativos.

Aspectos éticos en la investigación

El proyecto no requirió la captura o manejo de organismos. No se contemplan conflictos de interés.

Para la realización del proyecto se contó con el conocimiento y aprobación de las autoridades del Ejido Nuevo Becal, así como el permiso de trabajo y extracción de datos por parte de la Comisión Nacional de Áreas Naturales Protegidas y la Reserva de la Biósfera de Calakmul.

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Anexo I. Parásitos gastrointestinales identificados en muestras fecales de mono araña peninsular *(Ateles geoffroyi yucatanensis). Controrchis bibliophilus* (A), *Trypanoxyuris* sp. (B) y *Crypatosporidium* sp.(C).