Relationship between Toxoplasma gondii exposure and Forest Cover and Precipitation in Neotropical Primates of Costa Rica

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June 11, 2020

Abstract

The apicomplexan parasite Toxoplasma gondii has been found in more than 350 species of homoeothermic vertebrates in diverse climates and geographic areas. In most animals, T. gondii produces mild or asymptomatic infection. However, acute and hyperacute toxoplasmosis is associated with high mortality rates observed in Neotropical primates (NP) in captivity. NP are distributed in 20 countries across the Americas, and although infection has been reported in certain countries and species, toxoplasmosis in the wild and its impact on NP population survival is unknown. Differences among species in exposure rates and disease susceptibility may be due in part to differences in host behavior and ecology. Costa Rica has four species of NP, howler (Alouatta palliata), spider (Ateles geoffroyi), capuchin (Cebus imitator), and squirrel monkeys (Saimiri oerstedii). Here we report for the first time NP exposure to T. gondii using a modified agglutination test (MAT) in 245 serum samples of NP (198 wild and 47 from captivity) from Costa Rica. Associations of serostatus with environmental (forest cover, annual mean temperature), anthropogenic (human population density), and biological (sex) variables in howler and capuchin monkeys were evaluated. The seroprevalence among wild NP was 11.6% (23/198), compared with 60% (28/47) in captive monkeys, with significant differences between species (P <0.05), suggesting behavior and ecology influences. In general, antibody titers were low for wild NP (<128) and high for captive NP (>8192), suggesting higher exposure due to management factors and increased life span in captivity. Seropositivity in howler monkeys was positively related to forest cover and inversely related to annual rainfall. For capuchins, annual rainfall was inversely related to seropositivity. Surveillance of T. gondii in NP in captivity and in the wild is required to understand drivers of the infection and develop novel strategies to protect them.

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Short running title:

Toxoplasma gondii **in Neotropical Primates**

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Summary:

The apicomplexan parasite *Toxoplasma gondii* has been found in more than 350 species of homoeothermic vertebrates in diverse climates and geographic areas. In most animals, *T. gondii* produces mild or asymptomatic infection. However, acute and hyperacute toxoplasmosis is associated with high mortality rates observed in Neotropical primates (NP) in captivity. NP are distributed in 20 countries across the Americas, and although infection has been reported in certain countries and species, toxoplasmosis in the wild and its impact on NP population survival is unknown. Differences among species in exposure rates and disease susceptibility may be due in part to differences in host behavior and ecology. Costa Rica has four species of NP, howler (*Alouatta palliata*), spider (*Ateles geoffroyi*), capuchin (*Cebus imitator*), and squirrel monkeys (*Saimiri oerstedii*). Here we report for the first time NP exposure to *T. gondii* using a modified agglutination test (MAT) in 245 serum samples of NP (198 wild and 47 from captivity) from Costa Rica. Associations of serostatus with environmental (forest cover, annual mean temperature), anthropogenic (human population density), and biological (sex) variables in howler and capuchin monkeys were evaluated. The seroprevalence among wild NP was 11.6% (23/198), compared with 60% (28/47) in captive monkeys, with significant differences between species (P <0.05), suggesting behavior and ecology influences. In general, antibody titers were low for wild NP (<128) and high for captive NP (>8192), suggesting higher exposure due to management factors and increased life span in captivity. Seropositivity in howler monkeys was positively related to forest cover and inversely related to annual rainfall. For capuchins, annual rainfall was inversely related to seropositivity. Surveillance of *T. gondii* in NP in captivity and in the wild is required to understand drivers of the infection and develop novel strategies to protect them.

Keywords:

Serology, *Alouatta palliata* , *Ateles geoffroyi* , *Cebus imitator* , *Saimiri oerstedii,* endoparasites, Latin America

Main text:

Introduction

Toxoplasma gondii is an obligate intracellular parasite that can infect more than 350 species of mammals and birds worldwide (Dubey, 2010; Lindsay & Dubey, 2007; Tenter et al., 2000). It is the single species within its genus, in the phylum Apicomplexa (Roberts & Janovy Jr. 2000). The parasite (Dubey et al., 1970) undergoes sexual reproduction to produce oocysts in most domestic and wild felids, which function as definitive hosts (DH) and excrete oocysts in their feces. Oocysts are the infective stage for intermediate hosts (IH), and thus, felid latrines are a source of contamination for IH (VanWormer et al., 2013). *T. gondii* can also spread

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asexually, often via ingestion of encysted organisms in tissue (Su et al., 2003), allowing the parasite to bio-accumulate in IH such as carnivores and scavengers. In addition, several species of cockroaches, earthworms, and beetles function as mechanical hosts (Dubey, 2010; Chinchilla et al., 1994).

Seroprevalence of antibodies against *T. gondii* in hosts varies spatially (Afonso et al 2010) and temporally, and is influenced by climate (Salant & Spira, 2004; Afonso et al., 2010). For example, higher seroprevalence has been observed during years with high temperatures or high rainfall in humans (Hubálek, 2005), rabbits (Almeria et al., 2004), wild ruminants (Gamarra et al., 2008), and domestic cats (Afonso et al., 2006; 2010; 2013). Risk is often moderated through impacts on the distribution of domestic and wild cats, which are influenced by human factors such as the presence of prey, intra or inter-specific territorial interactions, environmental stress, vegetation, and topography (Horn et al., 2011).

Host-specific factors also influence susceptibility to *T. gondii*, with higher prevalence sometimes detected in larger rodents and lagomorphs than smaller ones (Afonso et al., 2007), and in species with a longer life expectancy (De Thoisy et al., 2003). This may be due to greater exposure to oocysts in species with larger home ranges, longer life expectancies, and higher energy requirements, which are related to body size (Ottaviani et al., 2006). Male cats have a five times greater chance of having antibodies than females (Afonso et al., 2007), possibly due to having larger body sizes and greater need to consume more and larger prey than females (Niewold, 1986). In French Guiana, burrowing, granivorous, and insectivorous mammals had much higher prevalences than arboreal ones (Carme et al., 2002). Mammals in terrestrial or mixed terrestrial / arboreal habits were more exposed to oocysts than strictly arboreal ones (Thoisy et al., 2003; Nardoni et al., 2011).

Among Neotropical primates (NP), the intensity of *T. gondii* pathology varies among infected species: such differences could be due in part to differences in ecology and behavior (Catão-Dias et al., 2013). Toxoplasmosis in the Callitrichinae NP subfamily (*Saguinus, Leontopithecus, Callithrix*) may cause almost 100% mortality, resulting in very low seroprevalence and contributing to difficulty in making ante-mortem diagnosis, particularly in free-ranging populations. NP in the *Saimiri* and *Aotus* genera of Cebidae and *Ateles* and *Alouatta* in Atelidae may experience acute and severe signs including prostration, anorexia, hypothermia, dyspnea, and vomiting, with mortality from 20-80%, allowing for seroprevalence of 15-66% (Catão-Dias et al., 2013). In contrast, signs among *Cebus* sp. NP are usually subacute and moderate, with a very low mortality rate that generates high and persistent IgG titers (Bouer et al 2010). Reports in captivity range from 28-79% of infected *Cebus* sp. monkeys, compared with 30.2% of animals in the wild (Garcia et al., 2005; Leite et al., 2008; Bouer et al., 2010). *Cebus* sp. NP may have evolved greater resistance than other NP species after frequent exposure to *T. gondii* (Catão-Dias et al., 2013). *Cebus* sp. NP commonly forage for insects on the ground and drink water from puddles and water holes, where they may encounter oocysts (Fragaszy et al., 2004). In addition, although most of the *Cebus* diet protein comes from invertebrates, they may consume a variety of vertebrates weighing up to 1/3 of their body weight and constituting up to 3% of their feeding time (Fragaszy et al., 2004).

In Costa Rica there are four species of NPs. While the Central American white-faced capuchin (*Cebus imitator*) and the Mantled howler monkey (*Alouatta palliata*) are considered at low risk (least concern) according to IUCN Red List, Geoffroy's spider monkey (*Ateles geoffroyi*) and both subspecies of the Central American squirrel monkey (*Saimiri oerstedii oerstedii* and *S. o. citrinellus*) are endangered mainly due to habitat loss and fragmentation (Cuarón et al., 2008a-e). Some species still are captured for the illegal pet trade, and are thus protected from international trade under Appendix I of the Convention on International Trade in Endangered Species (CITES, 2020). Although Costa Rica has protected areas that cover 25% of the country (González & Lobo, 1999), other conservation actions include a system of incentives to farmers known as payments of environmental services (PEP) since 1997 (Pagiola, 2008), and a national program of biological corridors (PNCB) to increase connectivity between forest patches, species migration and genetic flow (La Gaceta, 2006, SINAC, 2008). *Ex situ* actions include keeping some NP in zoos for education purposes and rescue centers across the country focus on rehabilitation and reintroduction of individuals back to protected areas.

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The high mortality rates observed in some NP can imperil already at-risk populations, but the causes of mortality and high rates of exposure are poorly understood (Bouer et al., 2010; Catão-Dias et al., 2013), so it is important to assess the infection status in captive NP. Living in altered environments or in contact with humans (where domestic cats are present) can affect NP behavior (McKinney, 2011) and exposure to infectious agents (Wolfe et al., 1998, Ekanayake et al 2004). Thus, it is important to evaluate impacts of *T. gondii* infection in NP in different areas that experience differential contact with human-altered habitat and have differing behaviors and diet (Fragaszy et al., 2004; Catão-Dias et al., 2013). Here we analyzed exposure to *T. gondii* in *A. geoffroyi, A. palliata, C. imitator and S. oerstedii* and the influences of environmental, anthropogenic, and biological variables in wild *A. palliata* and *C. imitator* in Costa Rica.

Materials and methods

Study area

The small tropical country of Costa Rica is located in southern Central America (latitudes 8 02'26' and 11 13'12'N, and longitudes 82 33'48"and 85 57'57"W) and has an area of 51,100 km^2 (with around 5% of the biodiversity in the planet). After having one of the world's highest deforestation rates during the 1970s, laws were changed and today, forested areas represent 52% of the country (MINAET and FONAFIFO, 2012), and 25% are protected (González & Lobo, 1999). The land's volcanic origin and its steep elevation gradients influence the country´s heterogeneity, which has 12 life zones distributed from intertidal zones to over 3000 meters above sea level (Holdridge & Grenke, 1971). Precipitation varies seasonally, being the highest from May to early November, and spatially, with wettest regions receiving over 7000 mm of rainfall per year, and the driest receiving around 900 mm (Avalos, 2019).

Sampling

This study used 245 serum samples from four species of native NP (*C. imitator*, *A. palliata*, *A. geoffroyi* and *S. oerstedii*) collected throughout Costa Rica in 2000-2015 as part of the project "Epidemiological, genetic, ethological and habitat studies in Costa Rica's monkeys" (Resolution 27-2013- National System of Conservation Areas, Figure 1.A.). Of the 245 NP, 198 were wild and 47 were in captivity. Most samples from captive animals came from three rescue centers, although 12 different sites were sampled. NP were anesthesized using IM injection of 3.3-11mg/kg of Zoletil 50® or 5-20 mg/kg ketamine with 0.5-2 mg/kg xylazine (Glander et al., 1991; Varela, 2006; West et al., 2007), which for wild NP was loaded into darts (PneuDart. Inc, Type P, 1cc) and delivered using a compressed gas rifle (PneuDart. Inc, model X-Caliber Gauged CO2) targeting the lateral triceps and quadriceps femoris (Glander et al., 1991). After an individual was anesthetized, blood was sampled from the femoral vein and placed into a cooler at 4°C. Once in the laboratory, samples were centrifuged at 2500 RPM for 5 minutes to separate serum, which was transferred to sterile 1.5ml tubes at -20°C until processed. Animals underwent a physical examination and were released upon awakening from anesthesia.

Serological analysis

A modified agglutination test (MAT) was used to detect immunoglobulin G (IgG) specific for *T. gondii* as previously described by Desmonts and Remington (1980), and Dubey and Desmonts (1987). Formalin-fixed tachyzoites were used as antigen (2x10^8 tachyzoites/mL from KeraFAST Inc., Boston, MA, USA. Catalog No. EH2002). Sera were serially diluted in buffered saline solution (1X PBS) from 1/4 to 1/8192 in 96-well round bottom microplates and 25 ul of antigen mixture (2.5 ml alkaline buffer, 35 ul 2-mercaptoethanol, 50 ul Evans blue dye (2 mg/ml in H$_2$O) and 150 ul *T. gondii* MAT antigen) were added to each well. The microplates were covered with sealing tape and incubated at 37°C for 24 hours and subsequently for 4 hours at 4°C. A positive reaction was characterized by a complete "mesh" of agglutination and a clear bottom, while sedimentation of antigen suspension in the form of a blue pellet at the bottom of the well was considered to be a negative reaction. Sera from mice and/or humans with a titer of 1/200 or greater were included as positive controls and negative controls were also included with each group of processed sera. All antibody titers that were 1/16 or higher were reported as positive. Titer values from captive versus free ranging primates were analyzed using a Mann-Whitney two tailed test to know if there are significant

differences at a 0.05 alpha value.

Biological, Environmental, and Anthropogenic Drivers

Due to insufficient sample size, data for *A. geoffroyi* and *S. oerstedii* were not included for further statistical analyses. Seven independent variables were explored for their association with exposure to *T. gondii* in *A. palliata* and *C. imitator* . Distance to villages, human population size, and human population density in the nearest village were considered indirect indicators of proximal domestic cats. The percentage of forest cover, annual average temperature, and annual precipitation were used as variables that affect oocyst survival. NP sex was used as an intrinsic factor for oocyst exposure (Table 1).

Forest cover, annual average temperature, and annual precipitation were measured within a 1.54 km^2 circular area (700 m radius) that was constructed around each sample´s geolocation point (QGIS software, version 2.14). Data for these variables were obtained from Costa Rica Digital Atlas (2014) and WorldClim (Hijmans et al., 2005). The buffer size aimed to include both species´ home ranges and adjacent areas (Wainwright 2007).

Generalized linear models (GLM) with binomial distribution were run in R V3.4.2 (R Core Team, 2013) to explore associations between predictor variables and *T. gondii* seropositivity. The original predictors (forest cover and precipitation) were centered by subtracting their means and scaled by dividing by their standard deviations. The most parsimonious model was chosen by selecting the one with the lowest Akaike information criterion (AIC). Akaike weights (wi) were calculated to determine the evidence in favor of each model and estimate the relative importance of variables (Table 2).

Each NP species was modeled separately. Multicollinearity among the seven variables was evaluated by means of the variance inflation factor (VIF). When analyzing all the proposed variables, a very high VIF value (>10) was obtained suggesting multicollinearity, therefore the model was reduced to four variables (sex, forest cover, annual precipitation, and human population density). With these models, none of the variables obtained a VIF value greater than 10 (Table 2).

Results

T. gondii -specific IgG antibodies were found in 59.6% (28/47) of captive NPs and 11.6% (23/198) of wild NPs. In captive NPs, seroprevalence was high for both species: 59.1% (26/44) of *A. geoffroyi* and 66.7% (2/3) of *C. imitator* . There were no significant differences in species or sex with 58.6% (17/29) of *A. geoffroyi* females and 60.0% (9/15) males being positive, while for *C. imitator* , 50.0% (1/2) females and 100% (1/1) male had antibodies (Table 3, Table 4).

In contrast, there was a significant difference in seroprevalence among wild NP species ($X^2 = 20,072$; $df = 3$; $P = 0.0002$): *A. geoffroyi* had the highest seroprevalence with 40% (2/5), followed by *C. imitator* with 30% (11/37) and *A. palliata* with 6.6% (10/151), while no *S. oerstedii* were positive. No significant differences were found regarding sex for any of the species, however, greater seroprevalence was observed for females in the three positive species (Table 3, Table 5).

Antibody titers tended to be relatively low (Median = 8) in wild NP (<128), with the exception of four individuals: one *A. geoffroyi* and two *C. imitator* with antibody titers of 262,144 and one *A. geoffroyi* with a titer of 1,048,576). In contrast, antibody titers were considerably higher (Median = 262144) in captive NP, from 8,192 to 33,554,000 (Table 6). These two groups of NP were statistically different in their titer values (Mann-Whitney U= 303, n1= 52, n2=34, p<0.00001 two tailed).

The model that best accounted for seropositivity among *A. palliata* (minimizing AIC) was forest cover and annual precipitation (Figure 1.B., Table 2), with a positive relationship between forest cover and seropositivity and inverse relationship with annual precipitation. However, evidence weights (wi) for the second and third best-supported models were 1.02 and 1.94 times lower than model 1 respectively, which suggests that no one model is strongly supported as the best candidate. The second best model was annual precipitation

(ΔAIC = 0.04) and the third was for human population density and annual precipitation (ΔAIC = 1.32). Notably, the precipitation parameter appeared among the top eight models.

For *C. imitator,* annual precipitation alone best accounted for seropositivity with a significant inverse relationship (Figure 1.C., Table 2). The next best models were forest cover and annual precipitation (ΔAIC = 1.09) and human population density and precipitation (ΔAIC = 1.5), with evidence weights 1.73 and 2.12 times lower than model 1, respectively. Again, the effect of precipitation on seropositivity was important for *C. imitator* when comparing the sum of the weights of Akaike (wi) in the models that contained this variable (0.98) with respect to the others, such as forest cover (0.35), population density (0.3) and sex (0.29).

Discussion

Our survey of *T. gondii* in NPs across Costa Rica documented widespread infection and species-specific risks of infection. The seroprevalence obtained for captive NPs is similar to other studies in South America, such as 30.8% and 76% using MAT in captive *Cebus apella,* (Leite et al., 2008; Pires et al., 2012), and 79% in*Cebus* sp. and 57% in *Ateles* sp. from zoos in São Paulo, Brazil (Bouer et al., 2010). The high prevalence of NPs in captivity may be due to management practices, including improperly washed fruits or vegetables, and raw or undercooked meat (Pires et al., 2012; Valentini et al., 2004), proximity of wild or domestic cats, and invasion of enclosures by infected birds and rodents that might be ingested by NPs (Pires et al., 2012; Valentini et al., 2004). These sources of infection would be similar for male and female NPs in captivity, consistent with our findings.

The 11.6% seroprevalence in wild Costa Rican NPs was lower than the 26.6% (n = 60) reported by Garcia et al (2005) using MAT in Brazil, and the prevalence we found for *A. palliata* (6.6%) is lower than documented for *Alouatta caraya* by Garcia et al (2005) in Paraná (17%, 3/17) and Molina et al (2014) in São Paulo, Brazil (75%, 15/20). However, it is very similar to findings by de Thoisy et al (2001), who found only 4% (2/50) seropositive *Alouatta senilicus* in French Guiana, and similar to this study had a higher proportion of positive females. Our data did not indicate a significant difference between sexes for any of the species, although this should be studied further given the sex bias observed in French Guiana and among domestic cats (Afonso et al., 2007).

In the case of *C. imitator* , the prevalence is equal to that reported by Garcia et al. (2005) in *Cebus* spp. with 30.2% (13/43). In fact, several studies have identified that carnivorous diet is a risk factor for *T. gondii* infection (Cabezón et al., 2011; Hejlicek et al., 1997). On the other hand, the prevalence found in*A. geoffroyi* (40%, 2/5) appears to be high, which is surprising considering its arboreal and herbivorous (frugivorous) behavior, characteristics that minimize its exposure to the parasite. No prevalence reports were found for wild *Ateles sp* . to compare this finding to. However, sample sizes for both *A. geoffroyi* and*S. oerstedii* were very small, so these findings should be interpreted with caution. The difference in seropositivity observed between howler (*A. palliata*) and capuchin monkeys (*C. imitator*) coincides with Garcia et al. (2005) and corresponds to behavioral characteristics between species. Howlers are predominantly folivorous, supplementing their diet with fruits, flowers and seeds, and obtaining most of the water they need from their food (reviewed by Wainwright, 2007). However, they can drink from water accumulated in branches, trunks or bromeliads (Glander, 1978; Gilbert & Stouffer, 1989), or search for it on the ground (Almeida-Silva et al., 2005). The source of infection for howlers would be water bodies infected with oocysts. Meanwhile, capuchins are the most omnivorous NPs, feeding on various sources like fruits, insects and small vertebrates such as birds, rodents, squirrels, coatis, bats, frogs and lizards (reviewed by Wainwright 2007 and Catão-Dias et al., 2013). They frequently go down to the undergrowth and ground while foraging and traveling (Feagle, 1999). In addition, *Cebus* spp. drink water directly from puddles (Fragaszy et al., 2004). These characteristics give capuchins greater exposure to oocysts in the soil / water or in invertebrates (transport hosts) and to tissue cysts present in infected vertebrates.

The low antibody titers obtained for the wild NPs (16 and 32) coincide with those reported by Garcia et al. (2005), who found mostly 16 and 32 for a single individual. While *A. palliata* had low antibody titers, two *A. geoffroyi* and two *C. imitator* had very high antibody titers (262,144 - 1,048,576). Molina et al

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(2014) reported values of 25 for *Callithrix penicillata* (a very susceptible species) and higher titers (up to 1600) for *A. caraya* , arguing that differences in prevalence and titers could respond to differences in host susceptibility, contact rates or post-exposure time. Indeed, *Alouatta* 's susceptibility in comparison to *Cebus* could be reflected in serological differences, since the probability of post-infection survival and, therefore, developing an immune response is naturally lower for howlers. Furthermore, if these individuals die, they would be excluded from the population and the sample, reducing the number of individuals captured with high titers. In contrast, titers of captive NPs were high in our study. Leite et al. (2008) reported antibody titers of 8000 by MAT for *Cebus sp* . in captivity, while Ekanayake et al. (2004) reported antibody titers >256 (up to 4096) in 3 of 21 positive free-ranging but urban macaques in Sri Lanka. Management factors along with the increased life expectancy for *Cebus* sp. and *Ateles* sp. in captivity could explain the chance of infection and high titers observed in this group.

It is worth highlighting the large number of positive samples from the Gulf of Nicoya (Figure 1.A.). Tempisque River is one of the most important basins in the country, draining 10.6% of the territory and flowing into the Gulf of Nicoya (Gutierrez et al 1985). Areas close to bodies of water could represent sources of infection for NP, because water can be contaminated at any point, transport oocysts long distances, and favor their survival (Lindsay & Dubey, 2009). Samples from protected areas with high levels of human contact also showed high prevalence and high titers in the two wild *C. imitator* mentioned before. In certain national parks and private reserves, feeding wild monkeys is a common practice, and capuchin and squirrel monkeys sometimes exhibit agonistic behaviors that include taking food directly from humans and coming down to the ground. Such opportunistic behavior (Campbell, 2013) may increase exposure to infectious agents (Wolfe et al., 1998; Ekanayake et al., 2004).

Besides identifying specific areas where there could be elevated risk of infection, our data indicates that environmental variables such as forest cover and precipitation could be associated with exposure risk. Seropositivity in *A. palliata* was higher when there was a higher percentage of forest cover and less annual precipitation. Forest cover can protect oocysts from sunlight, allowing them to remain viable where protected for 1-1.5 years (Ruiz et al., 1973; Frenkel et al., 1975). Smith & Frenkel (1995) and Almeria et al. (2004) found greater seroprevalence in hares and other mammals sampled in forested areas versus more arid grasslands, arguing that shadow and relative humidity provided by forest cover act on oocyst conservation by decreasing the evaporation rate and desiccation of oocysts.

Wet seasons tend to increase oocyst survival (Frenkel et al., 1975). In fact, *T. gondii* seroprevalence in cats (Afonso et al., 2013) and humans (Hubalek 2005) has been associated with rainy and warm episodes (North Atlantic Oscillation), and in wild ruminants (Gamarra et al., 2008) with humid areas. Contrary to expectations, in this study precipitation was inversely related with seropositivity in both NP species. Costa Rica is a tropical country with high relative humidity and stable temperature overall. It is possible that increased precipitation in Costa Rica results in greater runoff, transporting oocysts towards the coasts and away from the animals. In recent past years, low rainfall due to the El Nino phenomenon has generated severe droughts and forest fires in some areas of Costa Rica. Among many other animals, *A. palliata* were severely affected by water and food shortages, with high mortality due to dehydration and starvation, as well as injuries due to troops fighting for food. Because behavior change driven by droughts can increase exposure to parasites present in the scarce sources of water, the risk of disease increases especially for animals weakened by starvation and dehydration.

In the present study, the source of exposure of wild NP could be wild and not domestic cats, which might explain the low effect of human population density on seropositivity. Contact with humans has been associated with high seropositivity in macaques (Ekanayake et al., 2004), which can become infected by ingesting human food from the ground in areas frequented by domestic cats (Tenter et al., 2000). Domestic cat population estimates as well as sampling in areas where humans feed and interact with wildlife should be included in future studies.

Additionally, *Toxoplasma* genotypes produce different degrees of virulence in humans and mice (Xiao & Yolken, 2015), and given the high diversity recently found in Central and South America (Ajzenberg et al.,

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2004; Lehmann et al., 2004; Rajendran et al., 2012; Vitaliano et al., 2014; Rego et al., 2018; Vethencourt et al., 2019) the observed differences in seroprevalence between wild and captive NP might be due to different genotypes. Little is known about the genotypes circulating in wildlife, and associations between strain type, lesion patterns and clinical outcome have not been reported in wildlife frequently. Thus, future studies that focus on genotyping and virulence of *T. gondii* isolates in wildlife and domestic animals from wild and anthropized environments would be of great value.

In the present study, MAT was used as it is simple and fast, doesn't require specific modifications, can be used for large sample sizes and diverse host species, and can be run on serum and plasma (Desmonts & Remington, 1980; Shaapan et al., 2008; Dubey, 2010). An antibody titer of 1/25 is often considered as evidence of exposure to *T. gondii* in many mammals (Dubey et al., 1995) and 1/5 in birds (Dubey et al., 2016). However, there is no antibody titer that is considered specific for primates and our analysis required that we be able to maximize sensitivity of detection given the high susceptibility and low seroprevalence in some NP species, thus we reported all antibody titers of 1/16 or higher (Garcia et al., 2005).

Among the reagents used for MAT is mercaptoethanol which destroys immunoglobulin M (IgM) and therefore MAT only detects IgG (Seefeldt et al., 1989). Because IgG are maintained for life (Dubey, 2010), their detection indicates exposure to *T. gondii* at some point during the animal's life; inability to discriminate recent infections precludes assessing longitudinal shifts in seropositivity. However, IgG presence can be used to detect the spatial influences of environmental factors. Further analyses through longitudinal sampling and detection of IgM are recommended.

In conclusion, our study documented widespread *T. gondii* infection in NP and species-specific risks of infection for the first time in Costa Rica. The high seroprevalence and titers found in captive capuchin and spider monkeys may be due to management practices, the proximity of cats or intermediate hosts, and the increased life expectancy in captivity for these species. The low seroprevalence and titers in wild NP varied between species. The difference in seropositivity observed between wild howler (*A. palliata*) and capuchin monkeys (*C. imitator*) in this study agrees with behavioral and dietary characteristics, in which capuchins are more exposed to oocysts while foraging on the ground, and by ingesting invertebrates (transport hosts) and vertebrates (tissue cysts). However, *Alouatta*'s susceptibility compared to *Cebus* might also explain the observed serological differences, due to decreased survival. Our data indicated that specific areas could represent an elevated risk of infection (i.e. water runoff and human interaction), and environmental variables such as abundant forest cover and low precipitation could be associated with higher exposure risk in wild NP. Surveillance of *T. gondii* in NP is required to better understand the infection status, genotypes and drivers involved in wild and captive NP, including individuals in the process of reintroduction, so that biosecurity measures are improved, avoiding the release of infected individuals, and develop novel strategies to protect wild populations.

Acknowledgements

Ethics statement: The present study was conducted under the protocols established by the Institutional Committee for the Care and Use of Animals (Comite Institucional para el Cuidado y Uso de los Animales) of the Universidad de Costa Rica, adhered to the legal requirements of Costa Rica, and adhered to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates. Collection permit number: MINAET-SINAC-Costa Rica: 042-2012-SINAC.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions statement: C.N., and A.C. conceived the investigation and wrote the article, A.C., and G.G-E collected samples, C.N., N.R., and C.S., conducted the laboratory analysis, C.N., M. S., C.I-C., A.C. and O.R-C.: performed the statistical analysis, J.F., and G.S. copy reviewed and edited. All authors reviewed the manuscript.

Conflict of interest statement: The author(s) declare no competing interests.

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Table 1. Independent variables chosen for Generalized Linear Models (GLM) *Toxoplasma gondii* exposure in wild *Alouatta palliata* and *Cebus imitator* .

Table 2. Generalized Linear Models that explain intrinsic, environmental and anthropogenic attributes on *Toxoplasma gondii* seroprevalence in *Alouatta palliata* and *Cebus imitator* .

Table 3. Prevalence of *Toxoplasma gondii* antibodies in captive and wild Neotropical primates in Costa Rica according to species and sex. Samples of *Saimiri oerstedii* (n = 5) were excluded.

Table 4. Seroprevalence of *Toxoplasma gondii* in captive Neotropical Primates from Costa Rica according to site and species.

Table 5. Seroprevalence of *Toxoplasma gondii* in wild Neotropical Primates from Costa Rica according to sample site and species.

Table 6. Antibody titers of *Toxoplasma gondii* IgG in Neotropical primates of Costa Rica

Figure 1. Map of *Toxoplasma gondii* seropositive wild Neotropical primates (NP) from Costa Rica from 2000–2015: (A.) Protected areas, (B.) Forest Cover, (C.) Precipitation. Gray circles: seronegative NP, pink circles: seropositive NP.

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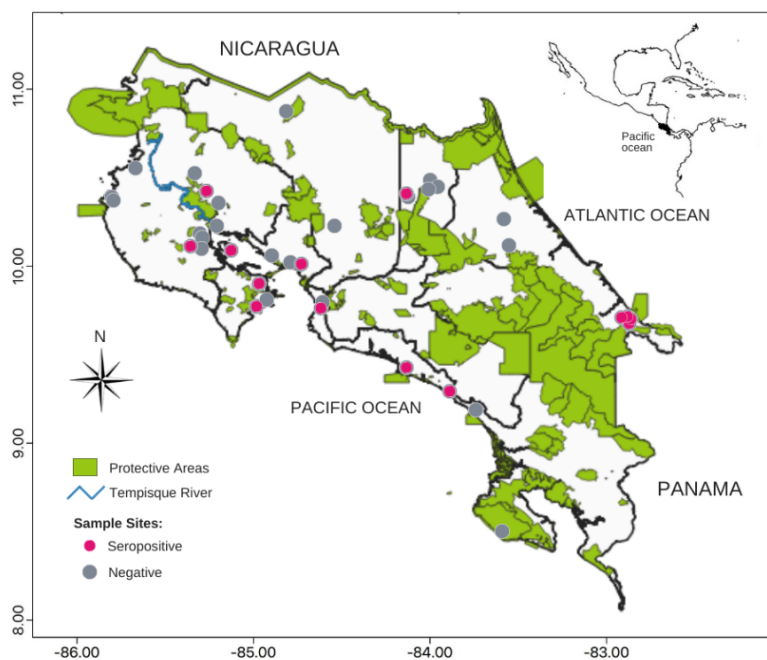
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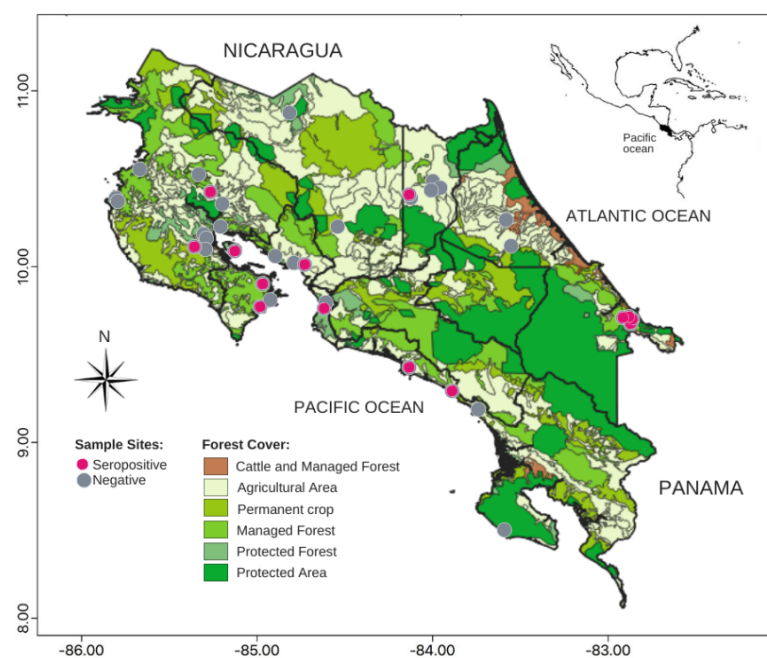
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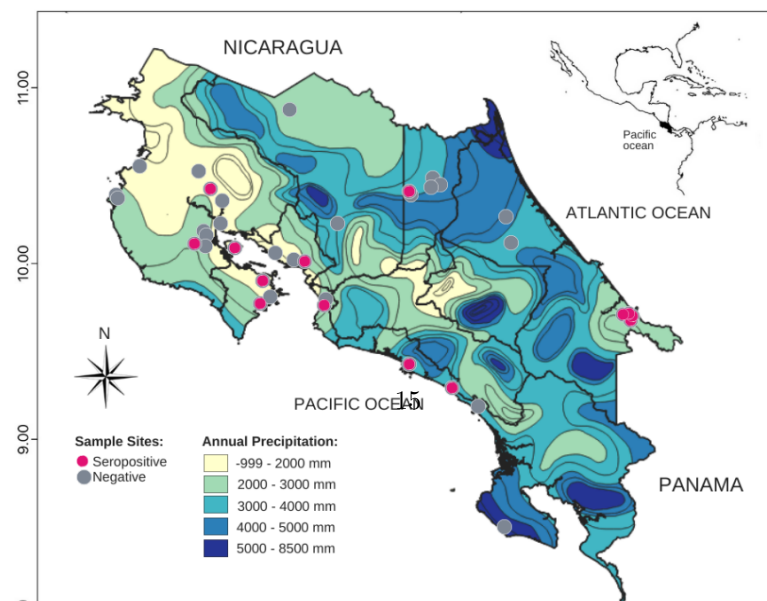
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A.

B.

C.