

## NATURAL LATENT TOXOPLASMOSIS IN WILD AND CAPTIVITY-BORN OLIVE BABOONS (*PAPIO ANUBIS*) IN KENYA

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## ABSTRACT

Toxoplasmosis is a neglected anthrozoosis caused by the obligate intracellular protozoan, *Toxoplasma gondii*. The role of non-human primates in the epidemiology of human toxoplasmosis is not clear. Acute and highly fatal cases of toxoplasmosis are frequently reported in new world monkeys and asymptomatic infections in old world monkeys. Here we report detection of latent natural *T. gondii* infection in olive baboons during a screening exercise to select animals for an experimental toxoplasmosis study. Archived serum samples from 32 olive baboons (*Papio anubis*), 23 wild caught and nine colony-born, were screened for *T. gondii* DNA using nested PCR. Eighteen (56.25%) samples were from females and fourteen (43.75%) from males. *Toxoplasma gondii* DNA was detected in 21 (65.6%) baboons. 13 females (40.6%) and eight males (25%). Five baboons (24%) which tested positive were from the Institute of Primate Research colony but there was no statistical significance between them and the wild trapped ( $p=0.453$ ). There was neither statistical significance ( $p=0.373$ ) between sex and infection status nor between area of origin and infection status ( $p>0.05$ ). These results indicate that olive baboons get infected with *T. gondii* in the wild and during captivity and may be significant reservoirs of human infections, especially where they may be trapped for bush meat. We recommend a country-wide study to establish true prevalence of toxoplasmosis among non-human primates and identify associated parasite strains.

*Key words: Toxoplasmosis, Toxoplasma gondii, nested PCR, non-human primates, Olive baboons*

## INTRODUCTION

*Toxoplasma gondii* is one of the most widespread parasites in the world, infecting about one third of the human population [1]. While domestic and wild cats are the only known definitive hosts, *T. gondii* infects virtually all warm-blooded animals which act as intermediate hosts. Humans become infected with *T. gondii* by ingesting tissue cysts from raw or undercooked meat [2], or by ingesting oocysts from contaminated food or water or directly from the environment. Most human infections are largely asymptomatic with only 10-20% exhibiting mild, self-limiting flu-like symptoms of lymphadenopathy and low grade fever [3]. Severe and potentially fatal disease usually result sequel to congenital infection and in immunosuppressed patients (e.g. HIV/AIDS) and those undergoing immunosuppressive therapy for conditions such as organ transplantation [4] and cancer treatment via radiotherapy and chemotherapy [5;6].

The role of non-human primates (NHPs) in the epidemiology of human toxoplasmosis has not been well elucidated, especially in Africa. Many reports are being made regarding toxoplasmosis in both wild and captive monkeys. New World primates are more susceptible to toxoplasmosis than their Old World relatives with some New World species being extremely susceptible to the disease, resulting in sudden outbreaks and deaths in colonies [7; 8; 9]. Only a few serological surveys have reported natural *T. gondii* infection in old world monkeys [10; 11; 12] none of which has reported clinical signs of toxoplasmosis. The first case of natural toxoplasmosis in free range Caribbean African green monkeys (*Chlorocebus sabaenus*) on the island of St. Kitts in the Caribbean was reported recently [13]. In South Africa, free-ranging Chacma baboons showed serological evidence of toxoplasmosis with some having tissue cysts in the heart, brain, and skeletal muscles, without any accompanying inflammatory response [14] suggesting that immunocompetent baboons develop latent toxoplasmosis just like humans. More recently studies have reported detection of anti-*T. gondii* antibodies in the Barbary macaque (*Macaca Sylvanus*) and one common chimpanzee (*Pan toglodytes*) in a Spanish zoo [12].

In Kenya, rapid human population growth has put wildlife-protected habitats under intense pressure through encroachment leading to Human Wildlife conflict (HWC). The establishment of wildlife conservancies, in non-protected areas, has also led to a closer relationship, between humans and wildlife which tends to intensify HWC). Small scale farmers continue to cultivate their lands as small islands surrounding large scale game ranches. Their crops are thereby exposed to continuous destruction by wildlife. In retaliation they engage in wanton poaching to subsidize their incomes. Nonhuman primates, which are trapped for bush meat, pose a great threat to human health by harboring zoonotic bacteria, viruses [15] and parasites [16]. A significant proportion of respondents in a study confessed involvement in bush meat trade and consumption but claimed they were not aware of the potential for disease transmission by the meat [17].

We hereby report that olive baboons get infected with toxoplasmosis in the wild and in captivity and may be significant reservoirs of human infections, especially in areas rampant with human-wildlife conflict where they may be trapped and used as bush meat.

## MATERIALS AND METHODS

**Ethical Clearance.** All protocols and procedures used in this study were reviewed and approved by the Institutional Animal Care and Use committee of the Institute of Primate Research in Kenya (approval number: IRC/21/11).

### Baboon Serum Samples

Thirty two archived serum samples from adult olive baboons (*Papio anubis*) were availed by the Institute of Primate Research (IPR), Kenya, to be screened for *Toxoplasma gondii* DNA prior to a toxoplasmosis infection study. Twenty three samples were from baboons trapped in the wild at Mutara ranch (10 baboons) in Laikipia County and Marura farm (13 baboons) in Aberdare regions (Nyandarua County) of Kenya. They were transported to IPR, Karen, where they were quarantined awaiting being used in various studies. Nine serum samples were from adult baboons (6 males, 3 females) born in the colony maintained at IPR (colony born). All the serum was archived at -20°C. Nanyuki County, at an altitude varying from 1,166 to 2,122m (3,825 to 6,962ft) above sea level, encompasses the high, dry Laikipia Plateau, has a cool, temperate climate with both rainy and dry seasons. Economic activity in the county consisting mainly of tourism and agriculture, chiefly grain crops, ranching and greenhouse horticulture. Environmental temperatures vary between 10-28 °C during wet and dry seasons respectively. The Aberdares consists of a heavily forested range, with average elevation of 12,000 to 13,000 feet above sea level with low temperatures ranging from 4-18 °C during wet and dry seasons respectively.

### Isolation of DNA and Screening of *T. gondii* by PCR

Genomic DNA was extracted from serum samples using a commercial kit (ZymoResearch Quick-gDNA kit, USA) according to the manufacturer's instructions. Detection of *T. gondii* infections was determined by nested PCR targeting the 529bp repetitive elements (Lau *et al.*, 2010). The PCR supermix consisted of 10x Taq buffer with 15mM MgCl<sub>2</sub> (Qiagen), 0.1 U Taq Polymerase, 0.2µl of 10mM dNTP mix, 0.15 µl of the 10 µM primers NF1 and NR1, and 6.5 µl ddH<sub>2</sub>O. 0.5 µl of extracted DNA was added to each labeled PCR tubes. The first PCR

amplification was performed at 2 minutes at 94°C followed by 30 cycles of 94°C at 1 minute, 60°C at 30 seconds and 72°C at 40 seconds. A further extension step was done at 72°C at 4 minutes. The primers sequences for the primary amplification were, NF1 (5'-TGACTCGGGCCCAGCTGCGT-3') and NR1 (5'-CTCCTCCCTTCGTCCAAGCCTCC-3'). The product of the primary amplification was then diluted 1:100 and used as the template for the secondary amplification. Secondary amplification was performed at 94°C for 1 minute followed by 30 cycles of 94°C for 10 seconds, 52°C for 15 seconds, 72°C for 20 seconds then followed by further extension at 72°C for 4 minutes. The primer sequences for secondary amplification were, NF2 (5'-AGGGACAGAAGTCGAAGGGG-3') and NR2 (5'-GCAGCCAAGCCGGAAACATC-3'). The PCR amplification product was analyzed on 1.5% agarose gel stained with ethidium bromide and a product size 164bp was expected.

### Data Analysis

Baboons were categorized according to sex, area of origin and toxoplasmosis status (Table 1). Chi square statistics were computed using SPSS version 20 to determine significance between toxoplasmosis status and the independent variables. Statistical significance was considered when  $p < 0.05$ .

### Results

The distribution of the baboons according to area of origin, sex and infection status is as in the table below;

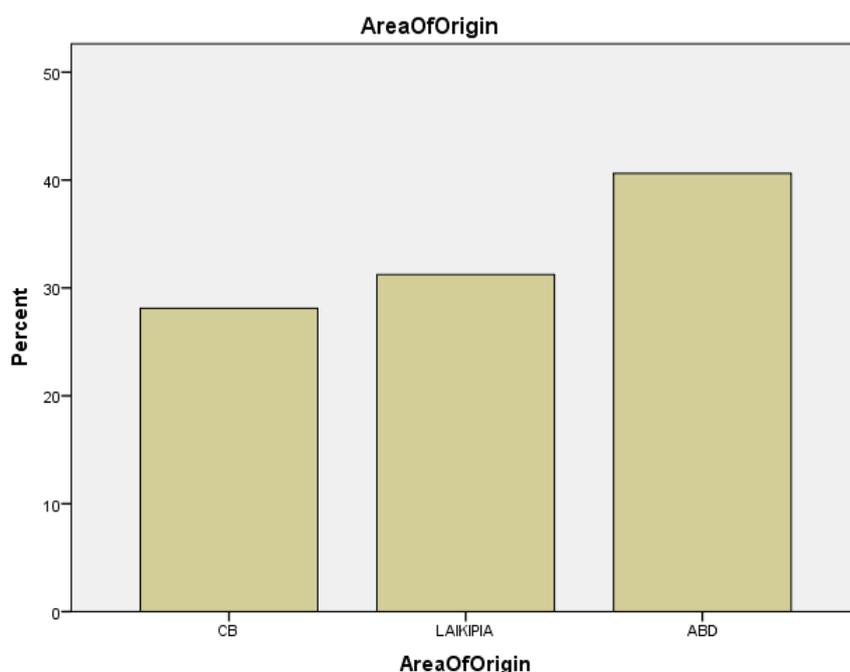
**Table 1. Percentage of baboons positive for *T. gondii* categorized by their area of origin and sex. ♂, Male; ♀, Female**

Area of Origin	Number Tested			Number positive			% Positive		
	♂	♀	Total	♂	♀	Total	♂	♀	Total
Colony Born	6	3	9	4	1	5	66.7	33.3	55.5
Laikipia	5	5	10	4	5	9	80	100	90
Aberdares	3	10	13	0	7	7	0	70	53.8

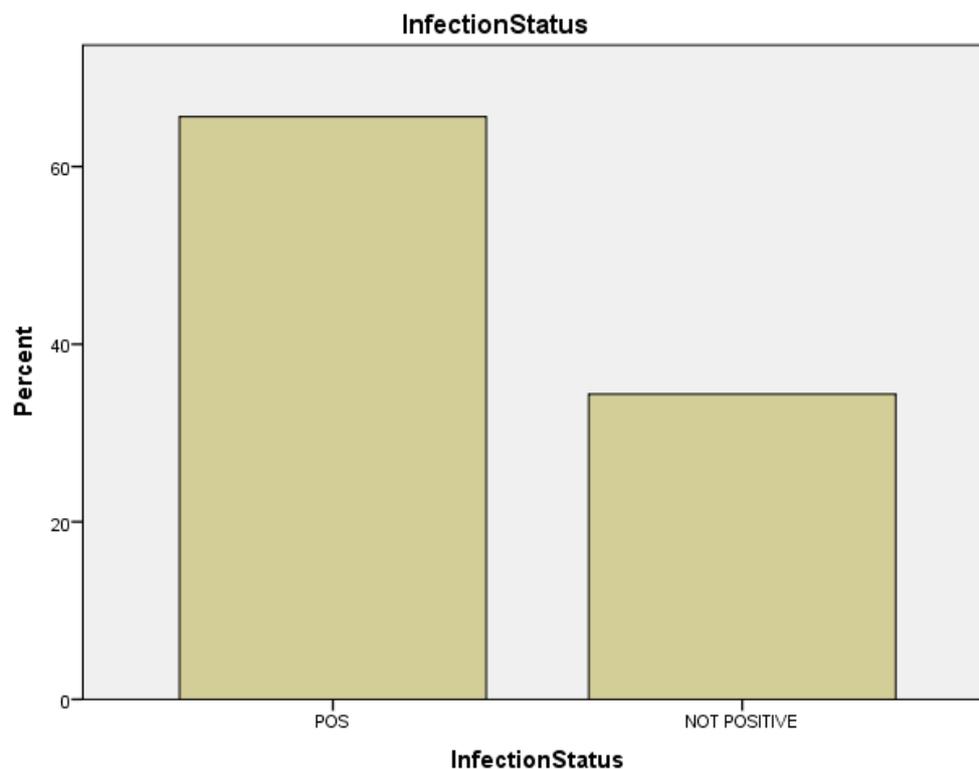
<b>Total</b>	14	18	32	8	13	21	57.1	72.2	65.6
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Out of the 32 screened baboons, twenty three (72 %) were trapped at Mutara ranch in Laikipia County, 13 at Marura farm in the Aberdares while 9 (28%) were born in the primate colony maintained at the Institute of Primate Research (IPR) as indicated in figure 1. Of the total 32 baboons tested, 18 (56.3%) were females while 9 (43.7%) were males. A total of 21 baboons (65.5%) tested positive, 13 (62%) females and 8 (38%) males.

There was no significant statistical relationship between sex and toxoplasma positivity status ( $\chi^2 = 0.7938$ ,  $df = 1$ ,  $p = 0.373$ ). Five baboons (24%), four males and one female, which tested positive were colony born. Statistical analysis also ruled out a significant relationship between positivity and area of origin of the baboons; colony born versus total wild trapped ( $\chi^2 = 0.563$ ,  $df = 1$ ,  $p = 0.453$ ), Colony born versus Laikipia ( $\chi^2 = 2.898$ ,  $df = 1$ ,  $p = 0.089$ ), Colony born versus Aberdares ( $\chi^2 = 0.0063$ ,  $df = 1$ ,  $p = 0.937$ ) and Laikipia versus Aberdares ( $\chi^2 = 3.489$ ,  $df = 1$ ,  $p = 0.062$ ).



**Figure 1. Distribution of baboons according to area of origin. CB, Colony Born, ABD, Aberdares**



**Figure 3. Percentage infection status of the baboons. POS, Positive infection status**

## DISCUSSION

To our knowledge, this is the first report of natural *T. gondii* infection in olive baboons (*Papio anubis*). Besides, it reports high prevalence rates of infection among both wild caught baboons and those born in a captivity. This suggests high contamination of the wild and captive environments with oocysts exposing the baboons to *T. gondii* infection. Wild and domestic cats are the only known definitive hosts for *T. gondii* shedding millions of oocysts into the environment. While some studies have indicated high prevalence of infection in female African green monkeys [13], pigs [18] and donkeys [19], this study does not show sex as a risk factor for occurrence of toxoplasmosis in olive baboons.

These results seem to suggest a likelihood of higher infection rates, though not statistically significant, ( $p=0.062$ ), in Laikipia than in Aberdares. This might be due to the fairly warmer environmental conditions in Laikipia (10-28°C) favoring better survival and sporulation of oocysts. Oocyst infectivity following sporulation optimally occurs at 11-25°C and favorable

aerobic and humidity conditions [20]. Furthermore, there is closer interaction between human settlements and wildlife in Laikipia since this is a cattle and game ranching area, making it possible for roaming domestic cats to contaminate the wildlife environment with more oocysts. Baboons are also known to invade human settlements during dry seasons in search of food and water increasing their chances of getting infected. Studies have reported that primates, which get in contact with human dwellings, have higher prevalence of toxoplasmosis than those that are restricted to their forestry habitat [10]. Since baboons are omnivorous, it is likely they acquire infection by consuming oocysts attached to vegetation. Consumption of insects and earthworms may also increase their chances of ingesting oocysts contaminating the soil. They may also get infected when they eat wild birds, hares, baby impalas and small antelopes [21] and domestic chicken and small goats. Free range chicken, especially, are known to be reservoirs of *T. gondii* tissue cysts [22]. Waterborne cases of toxoplasmosis have been described as well [23; 24]. Due to natural phenomena, oocysts are spread over considerable areas contaminating surface and ground water. They subsequently enter terrestrial systems through watersheds and eventually they are ingested by wildlife [25]. The high infection rates among baboons born in captivity may suggest the source of infection to be contaminated feed especially fresh vegetables and fruits though congenital infection may also be possible.

The role of the baboon in the epidemiology of human toxoplasmosis in Kenya is unknown. It is however important to note that unscrupulous persons have been apprehended selling baboon meat as game meat [17]. Furthermore where animals (including baboons) are killed due to human-wildlife conflicts, such animals are usually eaten by locals [17]. These scenarios may present a significant interface between human and wildlife toxoplasmosis. The findings of this study also seem to suggest that baboons, when used as models for the study of various parasitic infections especially protozoal, should first be screened for toxoplasmosis as they may provide erroneous results due to immunological cross-reactivity between the parasites [26].

### **Conclusions and recommendations**

We conclude that baboons are naturally infected by *T. gondii* in the wild and may be important reservoirs of parasites for human infection especially where they are trapped and used as bush meat. We also conclude that baboons in captivity get infected and control measures should be implemented to minimize their risk of exposure to *T. gondii*. We subsequently recommend a wide-scale study to determine the prevalence of *T. gondii* among nonhuman primates and identification of the prevalent strains. Epidemiological studies should also be carried out to determine the possible role of bush meat in human toxoplasmosis. All baboons used for biomedical studies should be routinely screened for toxoplasmosis.

### **Competing interests**

The authors declare that they have no competing interests

### **Authors' contributions**

DMK conceptualized the study and performed the nested PCRs. He also drafted the manuscript. SK, JK, MN and NM revised the manuscript. All authors read and approved the final manuscript.

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