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No evidence for yaws infection in a small-scale cross-sectional serosurvey in Ghanaian monkeys

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Abstract

Background: *Treponema pallidum* (*TP*) is a spirochaete bacterium with subspecies that in humans cause syphilis (subsp. *pallidum*), bejel (subsp. *endemicum*) and yaws (subsp. *pertenue*; *TPE*). The latter is target for eradication which requires detailed information on yaws epidemiology. It has been shown that African nonhuman primates (NHPs) are infected with *TPE* strains that are closely related to the human infecting yaws bacterium. While human yaws infection is known to be endemic in Ghana, there is a paucity of information regarding *TPE* infection of Ghana's native NHPs.

Objectives: The objective was to perform a small-scale cross-sectional serological screening for antibodies against *TPE* in Ghanaian monkeys. Due to the reports of *TPE*-infected NHPs from neighbouring Côte d'Ivore, we hypothesised that monkeys in Ghana are infected with *TPE* and, therefore, are seropositive for antibodies against-*Treponema*.

Methods: We sampled blood from 37 NHPs representing four species: *Erythrocebus patas* (16/37) 43.2%, *Papio anubis* (15/37) 40.5%, *Chlorocebus sabaeus* (3/37) 8.1% and *Cercopithecus mona* (3/37) 8.1%. Samples were tested using the NHP validated treponemal test ESPLINE TP.

Results: All 37 animals were seronegative for yaws infection.

Conclusions: We cannot exclude yaws infection in NHPs in Ghana at this point. Our study, in combination with the absence of reports of clinically infected NHPs in a yaws endemic country is, however, supportive for the current thinking that interspecies infection with *TPE* is extremely rare. This is an important finding for the current ongoing yaws eradication campaign.

KEYWORDS
bacterium, disease, serosurvey, spirochaete, *Treponema pallidum*, wildlife

1 | INTRODUCTION

Treponema pallidum (*TP*) is a spirochaete bacterium with subspecies that in humans cause syphilis (subsp. *pallidum*), bejel (subsp. *endemicum*) and yaws (subsp. *pertenue*; *TPE*) (Harper & Knauf, 2013). Yaws, a Neglected Tropical Disease, is a disfiguring chronic infection, which is usually prevalent in poor rural communities where children below the age of 15 are affected (Kazadi et al., 2014; Mitjà et al., 2012a). The implementation of the first global yaws eradication program between 1952 and 1964 reduced the global prevalence of yaws infection by approximately 95% (from 50 million to 2.5 million cases). Despite the huge reduction in prevalence, eradication of yaws was not achieved (Abdulai et al., 2018). As a result, between 2008 and 2012, over 300,000 new cases of yaws were reported to the World Health Organization (WHO), originating mainly from Ghana, Indonesia, Papua New Guinea and the Salomon Islands (Kazadi et al., 2014). With more than 100,000 reported cases between 2008 and 2015 (World Health Organisation, 2018), yaws is an important public health issue in Ghana. While treatment options with azithromycin (Mitjà et al., 2012b) have triggered the second, currently ongoing, yaws eradication campaign (Mitjà et al., 2015), recent studies in African nonhuman primates (NHPs) have raised concerns over a potential nonhuman disease reservoir. While our previous study demonstrated that NHP infecting strains belong to the subsp. *pertenue* (Knauf et al., 2018), confirming humans are not the exclusive host for the yaws bacterium, it is still unclear if NHP and human infecting yaws stains are epidemiologically connected. The first known NHP infecting *TPE* strain Fribourg-Blanc was isolated from a Guinea baboon (*Papio papio*) in West Africa in 1960 (reviewed in Šmajs et al., 2018). Infection was associated with mild keratotic skin lesions and ulcers on the muzzle, eyelids and armpits. More recent studies in the same geographic region report either baboon populations where serological positive animals had no signs of clinical disease (Knauf et al., 2015a) or an ongoing epidemic with skin ulcerated animals (Mediannikov et al., 2020). The latter is also seen in other regions of Africa (e.g. Tanzania), where high numbers of NHPs are infected (reviewed in Chuma et al., 2020). Similar to what is seen in humans, *TPE* induces a strong serological response in its NHP host (Knauf et al., 2015b). Since *TPE*-infected NHP populations generally show high seroprevalence rates independent of the presence or absence of clinical signs of infection, the detection of antibodies against *TP* has been proposed as a treponematosis screening tool for wild non-treated baboons (Knauf et al., 2015b). Even though Ghana is endemic for human yaws, there is a paucity of information when it comes to NHP infection. There is also limited data on the potential of NHPs as reservoirs for human yaws. This study aimed to investigate the *TPE*-infection status of NHPs in Ghana using an opportunistic cross-sectional serosurvey. Due to the reports of *TPE*-infected NHPs from neighbouring Côte d'Ivore (Knauf et al., 2018), we hypothesised that monkeys in Ghana are infected with *TPE* and, therefore, are seropositive for anti-*Treponema* antibodies.

2 | MATERIALS AND METHODS

2.1 | Study locations and animals

We performed opportunistic blood sampling of NHPs and included a total of 37 NHPs from different locations in Ghana (Mole National Park [Savannah Region; wild NHPs living near workers settlement] $n = 2$, Kumasi [Ashanti Region; captive NHPs at Kumasi Zoological Gardens and at Good Shepherd Preparatory School] $n = 6$, Shai Hills [Greater Accra Region; wild NHP troop at the entrance of Shai Hills Resource Reserve] $n = 8$, Tema [Greater Accra Region; pet monkeys] $n = 18$, Ashaiman [Greater Accra Region; pet monkeys] $n = 3$). The selection of populations and individuals concentrated on monkeys that live in close contact to humans. The animals represent four species: *Erythrocebus patas* (16/37) 43.2%, *Papio anubis* (15/37) 40.5%, *Chlorocebus sabaeus* (3/37) 8.1% and *Cercopithecus mona* (3/37) 8.1%. Further details can be found in Figure 1 and Table 1. All captive animals were wild born.

2.2 | Sampling of nonhuman primates

Blood samples from NHPs were taken according to the procedure described for our study in Tanzania (Chuma et al., 2018). Briefly, animals were chemically immobilised by remote distance injection by a cold gas immobilisation rifle (MOD JM; Dan-Inject ApS, Børkop, Denmark) with combination of ketamine (Ketavet, Pfizer, Berlin, Germany) (10 mg/kg body weight) and medetomidine (Domitor, Pfizer, Berlin, Germany) (0.2 mg/kg body weight). Animals were first weighed with a scale (KERNCH, Germany) before being placed in dorsal recumbency for sampling to commence. A physical examination of the NHPs was done and vital parameters such as temperature, respiratory rate, heart rate, pulse and blood oxygen saturation were taken every 5 min during sampling. The pulse, heart rate and blood oxygen saturation were monitored with a pulse oximeter (Oxi-Max N65, Nellcor, Malaysia). After disinfection of the skin, blood was sampled from the femoral vein using the closed collection system S-Monovette (Sarstedt, Nümbrecht, Germany), mounted with a 21G needle. For this study, up to one 9 ml K3 EDTA S-Monovette plasma tube and one 9 ml serum S-Monovette tube were filled per NHP sampled, depending on the animal's body weight. Subsequently, samples were cooled at +4°C and transported to the laboratory at Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) where plasma and serum was extracted by centrifugation at 2000 × *g* for 10 min. Aliquots were stored at −80°C until further processing.

2.3 | Serologic testing

The ESPLINE TP (Fujirebio Diagnostics, Hanover, Germany) treponemal test, which is validated for the detection of antibodies against *TP*

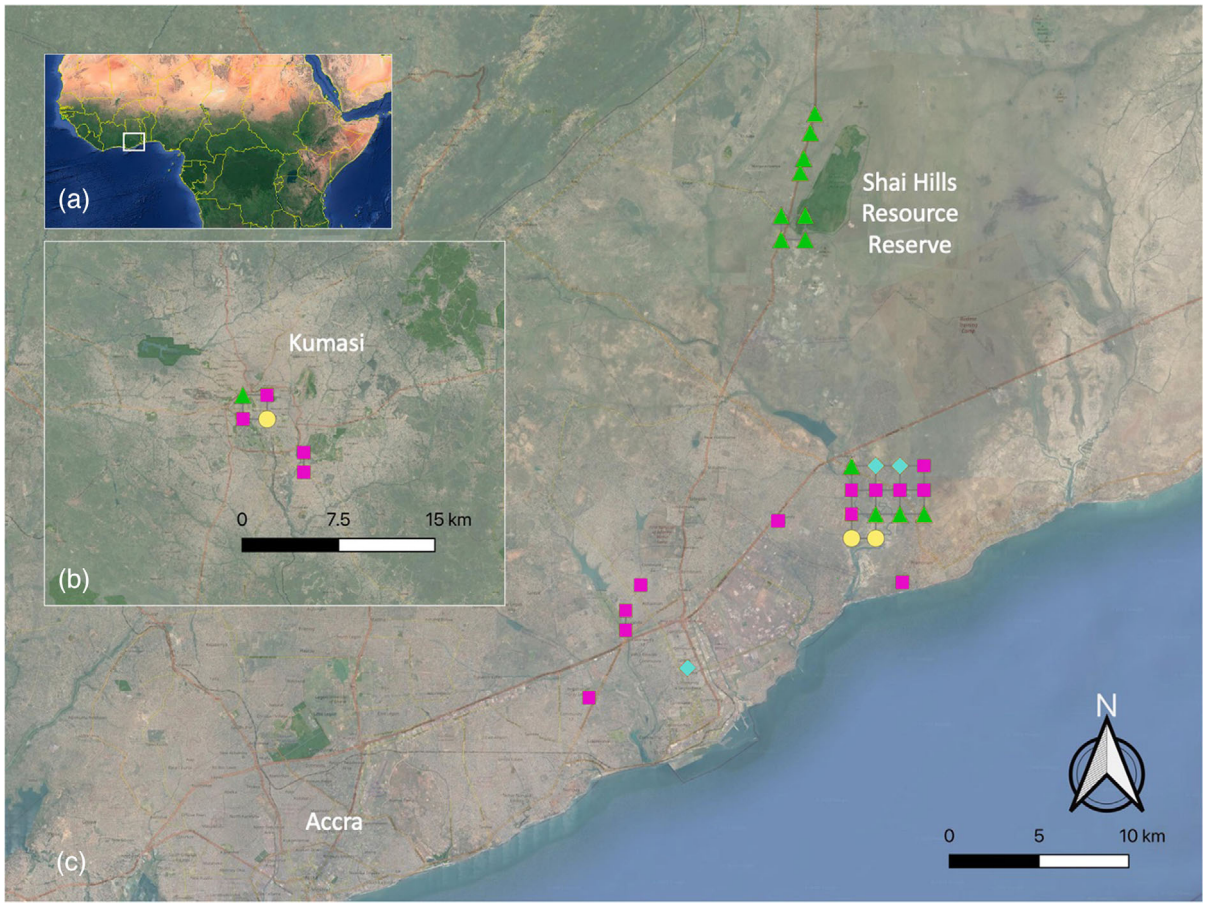

FIGURE 1 Sampling locations and distribution of NHP species sampled in this study. Overview of the sampling areas in Ghana, West Africa (a). Sampling locations in the Kumasi (b) and in the Accra area including Shai Hills Resource Reserve (c). Kumasi is located 200 km northwest of Accra. Symbols refer to different NHP species: circle = Mona monkey (*Cercopithecus mona*), rhomb = African green monkey (*Chlorocebus sabaeus*), square = patas monkey (*Erythrocebus patas*), triangle = olive baboon (*Papio anubis*). Animals sampled at close or identical locations are displayed as a grid. Only baboons sampled at Shai Hills Resource Reserve and Mole National Park were wild, while all other NHPs were kept in captivity (e.g. pet or zoo monkeys). Further details and the two wild NHPs which are missing GPS data can be found in Table 1. Maps were generated using QGIS version 3.20.0-Odense, map *source*: Google Satellite (https://mt1.google.com/vt/lyrs=s&x={x}&y={y}&z={z}) and Open Street Map (http://tile.openstreetmap.org/{z}/{x}/{y}.png)

TABLE 1 Summary of test results of infection in NHP samples in this study

NHP species	No. (%)	No. male	No. female	No. adult	No. subadult	No. young	ESPLINE TP results (Pos./Neg.)
Erythrocebus patas	16 (43.2)	5	11	4	7	5	Negative
Papio anubis[#]	15 (40.5)	11	4	9	3	3	Negative
Chlorocebus sabaeus	3 (8.1)	3	0	1	1	1	Negative
Cercopithecus mona	3 (8.1)	0	3	2	0	1	Negative
Total	37 (100.0)	19	18	16	11	10	

[#]For two of these individuals, GPS data are missing.

in NHPs (Knauf et al., 2015b), was applied according to the manufacturer's instructions. The test uses 25 μl of serum or plasma added to the sample window of the cassette with a sterile needle or pipette tip. Fifteen minutes after the test was started the results were read. The results were considered valid if the internal reference line was present (Knauf et al., 2015b).

3 | RESULTS

In our sampling set, distribution of males (M) and females (F) among the species were as follows: *Erythrocebus patas* ($M = 5$, $F = 11$), *Papio anubis* ($M = 11$, $F = 4$), *Chlorocebus sabaeus* ($M = 3$, $F = 0$) and *Cercopithecus mona* ($M = 0$, $F = 3$). All 37 sampled NHPs were free from any skin lesions that could indicate an active yaws infection and all animals tested negative for antibodies against *TP* (Table 1). Whenever possible we used serum samples to test for antibodies. In six cases, however, the serum samples were heavily haemolysed. In these cases, we used the K3 EDTA plasma sample to run the ESPLINE TP assay.

4 | DISCUSSION

Globally, more than 300,000 human yaws cases were reported to the WHO from endemic countries between 2008 and 2012 (Kazadi et al., 2014). The history of human yaws in Ghana goes back to 1936, when the burden of human yaws infection in humans constituted 62.7% of all infectious diseases treated in government health facilities (compared with 20.3% for malaria) (reviewed in Asiedu et al., 2014). Nearly a century later, yaws is still endemic in Ghana (Basing et al., 2020; Marks et al., 2017). Today, together with Papua New Guinea and the Salomon Islands, it accounts for 84.0% of the human yaws cases reported to the WHO. Between 2010 and 2011, the country recorded the highest number of yaws cases ($n = 20{,}525$) among the other known yaws endemic countries in the West African region, such as Côte d'Ivoire ($n = 3{,}704$), Togo ($n = 15$) and Cameroon ($n = 789$) (Mitjà et al., 2013). This information is complemented by a report that mentions a National Yaws Eradication Program Fact Sheet from 2017 (Okine et al., 2020), where Ghana had an average of 111 cases per 100,000 population per year and that yaws was found in all 16 regions of Ghana. The Central, Eastern and Volta regions were most endemic for human yaws, with a range of 5–338 cases/100,000 population over a 4-year period. The most recent data come from the year 2020, when Ghana reported a total of 4,695 suspected yaws cases (WHO, 2022). While it would have been ideal to sample (more) NHPs in the reported high endemicity regions for human yaws in Ghana, the sampling of NHPs was challenging and, therefore, had to be opportunistic. Compared to the situation in Tanzania (Chuma et al., 2018), wildlife in Ghana is generally shy and more elusive due to the regional high hunting pressure for bushmeat (Danquah et al., 2012). Remote distance injection was complicated by greater flight distance and mistrust of targeted animals. Nevertheless, we managed to include samples from the Southern edge of the Eastern Region (Shai Hills Resource Reserve; *Papio anubis* $n = 8$) and from the Ashanti Region, although only from captive animals (Kumasi; *Papio anubis* $n = 1$, *Erythrocebus patas* $n = 4$, *Cercopithecus mona* $n = 1$), which are both associated with notable numbers of human yaws infection (Marks et al., 2017). The challenges associated with sampling wild NHPs in Ghana are also reflected in the number of species that are represented in our data set. Currently, our data set contains only four out of the 17 NHP species that are found in Ghana (Mittermeier et al., 2013). Twelve of Ghana's 17 NHP species occur mainly in the high forest covers of the Brong Ahafo and the Western regions (Osei et al., 2015), areas where we have, as yet, not been able to sample NHPs.

Despite these obstacles, the results of this study suggest that *TPE* infection in Ghanaian NHPs is absent or occurs at a low prevalence. In theory and under the broader assumption that our 37 tested NHPs represent a random set of samples from a Ghanaian NHP population of unknown size, a documented ESPLINE TP test sensitivity of 97.7% as well as a specificity of 96.0% in NHP sera (Knauf et al., 2015b) the upper 95.0% confidence limit, using exact calculations, was determined at 9.48%, as calculated using propCI (package prevalence, http://prevalence.cbra.be/). This means that, statistically we should have detected infection in at least one animal in our sample set if the prevalence of *TP* infection in Ghanaian monkeys is greater than 9.5%. When using the same data to calculate the true prevalence (Blaker) (https://epitools.ausvet.com.au/trueprevalence) for all NHP samples ($n = 37$) and for olive baboons ($n = 15$) – a well-documented host species for *TPE* in sub-Saharan Africa that contributes most samples from wild individuals in this study – the calculated true prevalence has a 95% confidence interval (CI) of 0.00–0.06 and 0.00–0.17, respectively. In our previous studies (Chuma et al., 2018; Knauf et al., 2015a; Knauf et al., 2015b) and most of the investigations conducted by others (reviewed in Chuma et al., 2020; Knauf et al., 2015a), where the sample size was greater 30 animals, seropositivity rates in *TPE*-infected NHP populations in Africa were frequently higher than 9.5% in species belonging to the Cercopithecinae. The latter is a subfamily of the Old World monkey family Cercopithecidae, which includes monkey species tested in this study, which belong to genera that have previously been associated with *TPE* infection (Chuma et al., 2020). This background information, taken together with our findings, suggests that *TPE* infection is less likely to be present in our sampled Ghanaian NHP population(s).

While wild NHPs are generally not treated with antimicrobials, treatment in pet or zoo monkeys is common. However, even after a successful treatment of *TPE* infection, anti-*TP* antibodies should be present and detectable in NHPs. Under the One Health approach, yaws infection in primates is relevant not only for the eradication for human yaws. In areas of high endemicity in Ghana, there is a risk for primate species (e.g. baboons) that thrive in human-altered environments to acquire the infection from humans, a scenario that has also been deemed likely for seropositive pet macaques in Asia (Klegarth et al., 2017). There is also a conservation aspect to yaws infection in NHPs in Ghana, especially for the endangered species such as *Piliocolobus waldronae*, *Cercopithecus roloway*, *Colobus vellerosus* and the estimated 264 Western chimpanzees (*Pan troglodytes verus*) in the Bia-Goaso Forest Block in southwestern Ghana (Danquah et al., 2012). The latter is classified as critically endangered (Humle et al., 2016) and has recently been described to suffer from yaws infection in Guinea (Mubemba et al., 2020).

Up till now, there have been no reports of yaws lesions in Ghanaian NHPs. While our current study provides no evidence for NHP *TPE* infection in Ghana, it does raise new questions on the epidemiology of human and NHP infecting *TPE* strains. East African countries like Tanzania and Kenya were previously endemic with human yaws but lack current epidemiological data from human cases (Lubinza et al., 2020;

Zimmerman et al., 2019). Tanzania's last reported human yaws infections were in 1978 (WHO, 1984). NHP *TPE* infection, on the other side, has been reported in Tanzania since the 1980s. A recent study describes widespread NHP *TPE* infection in a majority of the national parks in Tanzania (Chuma et al., 2018). The yaws situation in Tanzania is, thus, in sharp contrast to our findings in Ghana. Unlike Tanzania, Ghana is still reporting human yaws cases and is one of the endemic countries for yaws in West Africa (Kazadi et al., 2014), while there is no current evidence for NHP infection in Ghana. It is possible that this situation is due to the fact that Ghana is one of the few countries in Africa with a national yaws programme that actively reports yaws cases in humans through the national health information system (Kazadi et al., 2014), while our current study is the first of its kind to investigate NHP infection in Ghana. Since Côte d'Ivoire shares a border with Ghana and is known to be endemic for human yaws (Kazadi et al., 2014), as well as reports of NHP *TPE* infections (Knauf et al., 2018) further investigations are warranted.

5 | CONCLUSION

We cannot exclude yaws infection in NHPs in Ghana at this point. Our study, in combination with the absence of reports of clinically infected NHPs in a yaws endemic country is, however, supportive for the current thinking that interspecies infection with *TPE* is extremely rare (Chuma et al., 2019). This is an important finding for the current ongoing yaws eradication campaign. However, posteradication surveillance is still necessary in Africa, where the potential for NHP-to-human infection remains based on the biology and close relationship of human and NHP infecting *TPE* (Knauf et al., 2018). NHP sampling in Ghana should be continued to reach a geographically equally distributed and species-specific sample size that allows a better statistical support to draw conclusions on the absence of *TPE* in Ghanaian NHPs.

AUTHOR CONTRIBUTIONS

The study was conceptualised by CR, AAS and SK. Field and laboratory work investigation was performed by EA, ISC, AAS and SK. Data curation and formal analysis were done by EA, AAS and SK. Funding acquisition, project administration and supervision were performed by CR, AAS and SK. EA, AAS and SK prepared the original draft manuscript. All authors reviewed and edited the script.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal as noted on the journal's author guidelines page have been adhered to. NHP samples were sampled with the permission of the Wildlife Division of the Forestry Commission of Ghana (Reference No. 0095189, project code: 01/01/2018) and according to their approved guidelines. Good Veterinary Practice rules were applied to all procedures when NHPs were handled for sampling. All procedures were conducted by a licensed veterinarian. Pet monkeys were sampled as part of routine health check-up in a veterinary clinic licensed by the Veterinary Council of Ghana.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are fully displayed in this article.

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