GENETIC CHARACTERIZATION OF *TREPONEMA PALLIDUM* ISOLATES AND DETECTION OF VIRUSES OF HUMAN HEALTH RELEVANCE IN FREE-RANGING NON-HUMAN PRIMATES OF TANZANIA

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EXTENDED ABSTRACT

Treponema pallidum is a group of non-cultivable spiral bacteria that cause treponematoses in humans in Europe and non-human primates (NHPs) since 1490s and 1960s, respectively. In humans, *T. pallidum pallidum* causes syphilis, *T. pallidum endemicum* is responsible for endemic syphilis and *T. pallidum pertenue* for yaws. The latter also infects various NHP species in Africa and elsewhere in the world. Historically, Tanzania is among 84 yaws-endemic countries that currently have little data available due to scanty research on treponematoses, both in humans and wild NHPs. In Tanzania, TPE infection has not been studied in NHPs other than olive baboons of Lake Manyara and Serengeti National Parks (Knauf, 2011; Harper *et al.,* 2012). Therefore, the current study was conducted from 2015 to 2017 across different ecosystems of Tanzania to investigate TPE infection in 289 free-ranging NHPs (eight species) and genetically characterize the TPE isolates. Using serologic treponemal test (Espline TP), this study detected anti- *T. pallidum* antibodies and showed that *Treponema pallidum* (TP) infection is geographically widespread in Tanzanian NHPs. The overall mean seropositivity was 53.3% (154/289) of which 60.7% (82/135) were females and males 46.8% (72/154) males. The NHPs tested included: vervet monkeys (77.8%, 35/45), olive baboons (85/137, 62.0%), yellow baboons (33/75, 44.0%) and blue monkeys (1/15, 6.7%). Three independent PCRs (*polA*, *tp47*, and *TP_0619*) confirmed these results but picked up 2 more positive cases missed by serology boosting the positivity to about 54% of NHPs (156/289) with four out of eight species testing positive at 11 of 14 locations. Majority of infected NHPs (59.8% ± 23.9% yellow baboons at 6 sites; 45.6% ± 16.2% olive baboons and 31.6% ± 9.4% vervet monkeys at 9 sites) had significantly more ($p<0.001$) anogenital ulcerations than orofacial lesions (3.5% olive baboons at Lake Manyara). Presence of antibodies against *T. pallidum* significantly associated with skin ulcerations in olive baboons ($p<0.0001$) and yellow baboons

(p=0.0185). Multi-Locus Sequence Typing (MLST) analysis of three genes (Tp0488, Tp0548 and Tp0619) revealed genetically diverse simian TPE strains in Tanzania and all the strains were closely related to TPE responsible for human yaws. Phylogenetic analysis showed geographical clustering of TPE strains, suggesting rare interspecies transmission. The strains had relative temporal stability and infection by multi-strain was evident. Antibiotic resistance was not found in Tanzanian NHPs. Serological analysis of randomly selected 74 NHPs using indirect immunofluorescence test (IIFT)-Chip technology (Euroimmun), detected antibodies reactive or cross reactive with 13 full viral antigens out of 20 that represent twelve virus families. These were: measles virus (89.2%, n=66), mouse hepatitis virus (78.4%, n= 58), mouse rotavirus (73.0%, n= 54), H1N1 Singapore (48.6%, n=36), yellow fever virus (37.8%, n=28), dengue virus (23.0%, n=17), adenovirus type 3 (21.6%, n= 16) and parainfluenza 2 virus (10.8%, n=8). None of the Tanzanian NHPs reacted with antigens from the rest seven viruses, including Ebola virus. Seropositivity of the NHPs to *T. pallidum* could was not linked to reaction or cross-reaction with any of the investigated viruses. More studies to further characterize simian and human pathogenic TPEs across Tanzania and Africa are highly recommended so as in the use of more specific tests in studies detecting and identifying simian viruses of human health significance.

DECLARATION

I, Idrissa Shomari Chuma, do hereby declare to the Senate of Sokoine University of Agriculture that this PhD Thesis is my own original work, independently done within the period of registration and that it has neither been submitted nor concurrently being submitted in any format to any other university or institution for a higher degree award.

10th July 2020

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Chuma, I. S., Abel, L., Hallmaier-Wacker, L. K., Šmajs, D. and Knauf, S. Pathogenic spirochetes in monkeys: stealthy pathogens of global importance. (Accepted by Springer Nature; Appendix 4).

PAPER I

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PAPER II

Chuma, I. S., Roos, C., Atickem, A., Bohm, T., Collins, D. A., Grillová, L., Hallmaier-Wacker, L. K., Kazwala, R. R., Keyyu, J. D., Lüert, S., Maloueki, U., Oppelt, J., Petrželková, K. J., Piel, A., Stewart, F. A., Šmajs, D. and Knauf, S. (2019). Strain diversity of *Treponema pallidum* subspecies *pertenue* suggests rare interspecies transmission in African non-human primates. *Scientific Reports* 9: 14243.

MANUSCRIPT II

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LIST OF ABBREVIATIONS AND SYMBOLS

Ab(s)	Antibody (Antibodies)
Ag(s)	Antigen(s)

ANP	Arusha National Park
CA	Conservation area
CCHFV-GPC	Crimean-Congo haemorrhagic fever virus
CDC	Centre for Disease Control and Prevention
CPXV	Cowpox virus
CVA24	Cocksakievirus A24
CVMBS	College of Veterinary Medicine and Biomedical Sciences
DBOV	Hantavirus
DENV	Dengue virus
DFU	Domain of unknown function
DNA	Deoxyribonucleic acid
Dnase	Deoxyribonuclease
DPZ	Deutsches Primatenzentrum (German Primate Centre)
DRC	Democratic Republic of Congo
EDTA	Ethylene diaminetetraacetic acid
EID	Emerging Infectious Diseases
ET	Ethiopia
F-B	Fribourg-Blanc (*T. pallidum* strain)
FTA-Abs	Florescent Treponemal Antigen Absorption
GNP	Gombe National Park
GPS	Global Positioning System
H1N1 Singapore	Parainfluenza virus
HAdV	Human Adenovirus
HcoV 229E	Human Coronavirus type 229E
HIV	Human Immunodeficiency Virus
IV	Intravenous

KNP	Katavi National Park
LACV	La Crosse virus
LAMP	Loop-mediated isothermal amplification PCR
LBS	Lyme borreliosis spirochetes
LMNP	Lake Manyara National Park
MAT	Microscopic agglutination tests
Mb	Megabase
MeV	Measles virus
MKNP	Mikumi National Park
MLST	Multilocus sequence typing
MNP	Mahale Mountains National Park
MSM	Men having sex with men
MU	Million units
MVH-1	Mouse hepatitis virus type 1
MVM	Minute virus of mice
NCA	Ngorongoro Conservation Area
NCAA	Ngorongoro Conservation Area Authority
NHP(s)	Non-human primate(s)
NIMR	National Institute for Medical Research (Tanzania)
NP	National Park
NWM(s)	New world monkey(s)
OWM(s)	Old world monkey(s)
PCR	Polymerase chain reaction
PIV 2	Picornavirus type 2
PPEs	Personal protective equipment
Prof(s)	Professor(s)

RC	Republic of Congo
RNA	Ribonucleic acid
RNP	Ruaha National Park
rpm	Revolutions per minute
RPR	Rapid plasma reagin
rRNA	Ribosomal RNA
RVFV	Rift valley fever virus
SEM	Standard error of the mean
SeV	Sendai virus
SGR	Selous Game Reserve
SIV	Sindbis virus.
SNP	Serengeti National Park
SPHS	Severe pulmonary hemorrhage syndrome
Spp.	Species
STD	Sexually transmitted disease
SUA	Sokoine University of Agriculture
Subsp.	Subspecies
TANAPA	Tanzania National Parks
TAWA	Tanzania Wildlife Management Authority
TAWIRI	Tanzania Wildlife Research Institute
TBEV	Tick-borne encephalitis virus
TEN	*Treponema pallidum* subspecies *endemicum*
TNP	Tarangire National Park
TP	*Treoponema pallidum*
TPA	*Treponema pallidum* subspecies *pallidum*
TPE	*Treponema pallidum* subspecies *pertenue*

TPeC	*Treponema paraluisleporidarum* ecovar Cuniculus
TPeL	*Treponema paraluisleporidum* ecovar Lepus
TPHA	*Treponema pallidum* hemagglutination
TPPA	*Treponema pallidum* particle agglutination
TZ	Tanzania
UG	Ugalla (Issa Valley)
UMNP	Udzungwa Mountains National Parks
URT	Upper respiratory tract
USA	United States of America
VDRL	Venereal Disease Research Laboratory
WHO	World Health Organization
WU	Washington University
YFV	Yellow fever virus
ZEBOV	Ebola virus

CHAPTER ONE

1.0 Introduction

Treponema pallidum is a spirochetal bacterium of worldwide importance. This bacterium has three recognized subspecies that cause diseases in humans, namely *Treponema pallidum pallidum* (TPA), which causes syphilis, *Treponema pallidum pertenue* (TPE), a causative agent of yaws and *Treponema pallidum endemicum* (TEN), which causes endemic syphilis (Kapembwa 2009; Knauf, 2011). According to Sefton (2001), the origin of treponematosis in humans, specifically syphilis, is controversial and dates back to 1493, when the first outbreak of syphilis spread across Europe. Pre-Columbian theory states that syphilis was present in Europe before the return of Columbus and his mercenaries from the New World (Sefton, 2001). This is contrary to the most famous Columbus theory, which implies that syphillis originated from the Americas as the first epidemic broke out upon the return of Columbus and his crew from the New World. The Evolutionary or Unitarian theory postulates that different members of the genus Treponema evolved from a single organism that manifest differently as a response to changes in the environment (Hudson, 1963).

T. pallidum subspecies cannot be distinguished based on serology; rather, they are differentiated by clinical characteristics and geographic distribution as well as genetic analysis detecting various genetic signatures (Flasarová *et al*., 2012; Happer *et al*., 2012; Knauf *et al*., 2012, 2013; Mitja *et al.*, 2013b; Chi *et al*., 2015; Chuma *et al*., 2019). In humans, TPA has adapted to venereal transmission with worldwide distribution and ability to cross the placental barrier resulting into congenital infection (Nathan *et al.*, 1997; Richens and Mabey, 2009). Non-venereal strains (TPE and TEN) cause skin lesions during early childhood in humans (Antal *et al.*, 2002; Kapembwa, 2009). Yaws is directly transmitted by skin contact predominantly in developing countries with warm and humid

climate in Africa and Asia, whereas endemic syphilis occurs in arid regions (Mikalová *et al.*, 2010; Knauf *et al.*, 2013).

Non-human primates (NHPs) in Africa are naturally infected with *T. pallidum* and the first reports were published between 1960s and 1970s (Fribourg-Blanc *et al.*, 1963, 1969; Fribourg-Blanc and Mollaret, 1969; Felsenfeld and Wolf, 1971; Baylet *et al.*, 1971a; 1971b). Treponematoses have been reported in monkeys and great apes along the tropical belt of Africa (Wallis and Lee, 1999; Levréro *et al.*, 2007; Harper *et al.*, 2012*;* Harper and Knauf, 2013). Although recent data show that all simian strains are closely related to human yaws-causing TPE strains, clinical signs of the disease in NHPs differ geographically (Harper *et al.*, 2008; Knauf, 2011; Harper *et al.*, 2012; Zobaniková *et al.*, 2013). In West African Guinea baboons (*Papio papio*), the clinical signs were mild, consisting of small lesions around the muzzle, eyelids and armpits but most animals had no clinical signs at all (Fribourg-Blanc and Mollaret, 1969). A recent survey in 2013 by Knauf and others (2015a) detected antibodies against the spirochete at Parc National du Niokolo-Koba, Senegal. This shows that clinically healthy Guinea baboons are infected even 30 years after first description of *T. pallidum* in NHPs by Fribourg-Blanc.

On the other hand, severe yaws-like lesions in tertiary-stage were reported in wild gorillas (Levréro *et al.*, 2007). Recently, *T. pallidum* infections with severe genital lesions with syphilis-like characteristics have been reported in wild olive baboons (*Papio anubis*) in East Africa (Knauf, 2011; Harper *et al.*, 2012; Knauf *et al.*, 2013). These findings have increased attention to further understand simian *T. pallidum* strains in East Africa.

1.1 Problem Statement and Justification

The World Health Organization's (WHO's) first yaws eradication campaign in the mid-20th century, was tremendously successful in reducing cases by 95% but failed to eradicate the disease (Maurice, 2012). High prevalence of NHP infection in areas where yaws is common in humans in tropical Africa suggests that cross-species infection may occur (Knauf *et al.*, 2013). The West African simian of *T. pallidum* strain can cause sustainable infection in humans, and laboratory infections of NHP with human isolates have been reported (Sepetjian *et al.*, 1969; Smith *et al.*, 1971). Non–sexually transmitted *T. pallidum* subspecies *pertenue* and *T. pallidum* subspecies *endemicum* (responsible for yaws and endemic syphilis, respectively) and sexually transmitted *T. pallidum* subspecies *pallidum* (causative agent for syphilis) are closely related (Knauf *et al.*, 2013). Genetic studies have also shown that spirochetes infecting baboons in West and East Africa do closely resemble *T. pallidum* subspecies *pertenue* (Harper *et al.*, 2008, 2012; Knauf, 2011; Zobaniková *et al.*, 2013). Recent whole genome analysis by Zobaníková and others (2013) was concluded with a proposal to reconsider the Fribourg-Blanc strain of *T. pallidum* isolated from a baboon in Guinea as *T. p.* subspecies *pertenue* strain Fribourg-Blanc.

In Tanzania, where no human yaws cases have been reported since 1982, when the last 78 cases were reported (Berger, 2017), a recent genetic characterization of *T. pallidum* strain responsible for genital disease in baboons of Lake Manyara National Park (LMNP) revealed its close relatedness to human yaws-causing strains (Knauf, 2011). However, the strain differs from another *T. pallidum* isolate from baboons in Serengeti National Park, SNP (Harper *et al.* 2012; Knauf *et al.*, 2012). The SNP and Ngorongoro Conservation Area (NCA) are neighbouring protected areas in the Serengeti ecosystem, which is about 50km away from Tarangire-Manyara ecosystem, where LMNP is. It is also well known that baboons in Gombe National Park (GNP) are infected with *T. pallidum* (Wallis and

Lee, 1999). Gombe is about 700km away from Tarangire-Manyara and Serengeti ecosystems and is the first area in Tanzania where *T. pallidum* infection in baboons was reported. In conclusion, genetic and epidemiological attributes of *T. pallidum* and treponematoses in free-ranging NHPs and their relationship to human yaws in Africa are not well understood. Such a gap might have impeded the first WHO's global yaws eradication campaign in mid 20th century and potentially also affects the on-going second WHO campaign to eradicate yaws globally by 2020.

Major yaws foci are in the Pacific, West Africa and Southeast Asia regions with 13 countries being endemic and actively infected; however, little epidemiological information is available from 84 other countries that historically were yaws-endemic (Marks *et al.,* 2015). Several studies on *T. pallidum* infection have been undertaken in West African primates but research in wild primates of East Africa is scanty (Harper *et al.,* 2012). With the exception of studies on olive baboons at LMNP and SNP (Knauf, 2011; Harper *et al.,* 2012), genetic characterization of *T. pallidum* species and strains infecting other NHP species in Gombe and other areas in Tanzania has not been done. Additional sequencing and genetic characterization of *T. pallidum* strains collected from humans and baboons as well as other affected NHP species, such as patas monkeys (*Erythrocebus patas*), gorillas (*Gorilla gorilla*) and chimpanzees (*Pan troglodytes*) (Fribourg-Blanc and Mollaret, 1969; Kuhn, 1970) could further clarify the relationship between human and NHP strains (Harper *et al.,* 2012).

Increase in scale and speed of mapping yaws is important to enable successful eradication (Marks *et al.,* 2015). In line with these recommendations, the current study identified, genetically characterized and mapped *T. pallidum* species and strains that infect several free-ranging NHP species in different ecosystems of Tanzania. This study was undertaken

as an effort to understand the relationship between simian and human treponematoses and fill this gap of scientific information. As the treponematoses negatively impact the tourism sector, this study was of special interest to Tanzania government and wildlife conservation authorities, especially the Tanzania National Parks (TANAPA), Ngorongoro Conservation Area Authority (NCAA), Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Wildlife Management Authority (TAWA). The authorities were in need to understand treponematoses in free-ranging NHPs in protected areas under their jurisdiction. In a wider perspective, scientific data generated by this study may be useful in formulation of strategies and management interventions for efficient control and eradication of treponematoses worldwide. Also, the study may positively contribute to address the missing link in the evolution of syphilis.

1.2 Research Questions

The following were the key research questions that lead to the objectives of this study:

a) Is treponematosis a common disease of free-ranging NHPs in Tanzania and how far has the disease spread among free-ranging NHPs?

b) What *T. pallidum* species and strains cause treponematosis in NHPs of Tanzania?

c) How are simian *T. pallidum* species and strains genetically related across Tanzania and how are they compared to human yaws-causing TPE strains?

d) What viruses of human health relevance infect free-ranging NHPs in Tanzania?

1.3 Objectives of the study

1.3.1 Overall objective

The main objective of this study is to characterize *T. pallidum* and detect viruses of human health relevance that infect free-ranging NHPs in wildlife protected areas within different ecosystems of Tanzania.

1.3.2 Specific objectives

a) To determine distribution and host species composition of *T. pallidum* infection in free-ranging NHPs in Tanzania
b) To characterize *T. pallidum* isolates from free-ranging NHPs in Tanzania using multi locus sequence typing (MLST) technique
c) To determine viruses of human health relevance that infect free-ranging NHPs in Tanzania and establish whether there is association these viruses and *T. pallidum* infection

1.4 Study Design and Sample Size Estimation

A cross-sectional survey with random, purposive and opportunistic invasive and non-invasive sampling of NHPs was undertaken in protected areas, within six ecosystems with different ecological attributes in Tanzania (Fig.1). Several criteria were considered in selection of the ecosystems and sampling sites including: presence of the NHP species, geographical and/ecological conditions, accessibility, NHP group behaviour, interactions between baboons and other NHP species and between the NHPs and humans. The sample size was estimated as explained by Thrusfield (1995) in the formula:

$$n = Z^2 p\,(1-p)/\,d^2 \quad \text{.....(i)}$$

Where: n is sample size; Z is the multiplier from the normal distribution (Z=1.96), p is the expected prevalence of *T. pallidum* infection in NHPs (p=0.11; Mlengeya, 2004) and d is the desired absolute precision (d=0.05). A minimum sample size of 150.4 was increased by 8% to 162 individual NHPs. A total of 289 free-ranging NHPs across Tanzania were chemically immobilized, sampled and released back to the wild. As the infected olive baboons do directly or indirectly interact with other NHPs, it was assumed that NHPs, other than olive baboons, are equally infected with *T. pallidum*. Therefore, the NHP species involved in this study were: 137 olive baboons (*Papio anubis*) yellow baboons

(*Papio cynocephalus*), 44 Vervet monkeys (*Chlorocebus pygerythrus* ; Blue monkeys (*Cercopithecus mitis*), 10 Zanzibar red colobus (*Piliocolobus kirkii* Udzungwa red colobus (*Piliocolobus gordonorum*), 2 red tailed monkeys (*Cercopithe* *ascanius*) and 2 Uganda or Ashy red colobus (*Piliocolobus tephrosceles*).

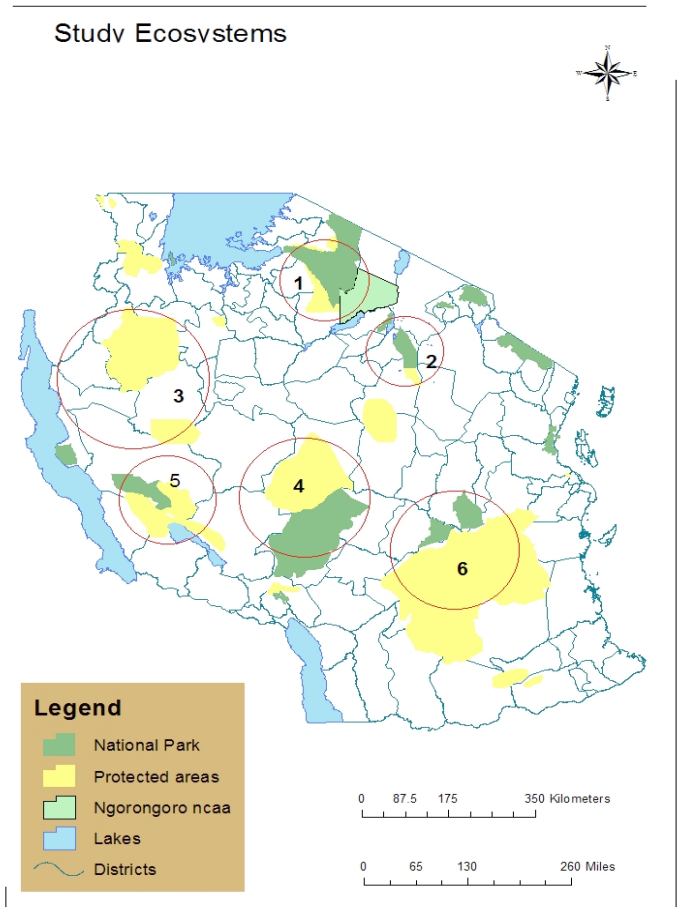

Figure 1: A map of Tanzania showing study ecosystems (in red circles)

1 Serengeti, 2 Tarangire-Manyara, 3 Ugalla-Moyowosi, 4 Ruaha-Rungwa, 5 Katavi-Rukwa and 6 Selous-Mikumi. (TANAPA GIS Unit®).

1.5 Literature review

1.5.1 Treponemes

Treponemes are bacteria classified under phylum Spirochaetae, order Spirochaetales, class Spirochaetes and genus Treponema, together with two other closely related genera namely

Borrelia and Leptospira. Treponemes are Gram-negative bacterium, motile, small 6-15 μm long and 0.1 - 0.2 μm wide spirochaetes, helically or spirally coiled either cultivable or non-cultivable bacteria with cockscrew-like shape. These bacteria have inner cytoplasmic membrane, which is loosely enclosed by outer membrane (Engelken *et al.,* 1991a; LaFond and Lukehart, 2006) with fewer integral outer membrane proteins among which some have been identified as targets for opsonic activity (Izard *et al.,* 2009; Liu *et al.,* 2010). They have a thin layer of peptidoglycans that anchor the cytoplasmic membrane to the outer membrane for cellular stability (LaFond and Lukehart, 2006). The endoflagellae located within the periplasmic space between the two membranes enable them to perform a characteristic corkscrew motility (Jepsen *et al.,* 1968).

Treponemes can also be classified as cultivable and non-cultivable pathogens basing on whether they can grow on artificial media/ cell culture or not. They also may be further categorized as pathogenic or non-pathogenic in accordance with ability to infect and cause diseases (*treponematoses*) in humans, domestic and wild animals in different parts of the world. The alternative grouping is based on mode of transmission of the diseases they cause (with name of the disease in brackets), which further stratify these spirochaetes into venereal transmitted *Treponema pallidum* subspecies *pallidum*, TPA (syphilis) and non-venereal transmitted ones including *T. pallidum* subspecies *pertenue*, TPE (yaws); *T. pallidum* subspecies *endemicum*, TEN (bejel) and *Treponema. carateum* (pinta) that are responsible for endemic treponematoses in various parts of the globe (Knauf *et al*., 2013).

1.5.2 *Treponema pallidum* infection

Treponema pallidum infects humans, wild and domestic animals as discussed in details in the following paragraphs:

1.5.2.1 Human infection

Syphilis, yaws, endemic syphilis and pinta are all multistage diseases with different transmission modes and clinical signs. These three diseases cause chronic infections that initially appear as single lesions and the entry point but later on spread to invade tissues including skin, cartilages and bones (Lukehart and Giacani, 2014). Syphilis is mainly a venereal transmitted disease though vertical transmission from an infected mother to child is also possible. Yaws and endemic syphilis are basically skin diseases respectively spread by direct skin-to-skin and mucous membrane contacts, though indirect transmission is also possible via shared objects like utensils (Hudson, 1938; Arslanagic *et al.*, 1989; Giacani and Lukehart, 2014). Involvement of fly vectors have also been suggested for yaws (Kumm and Turner, 1936; Satchell and Harrison, 1953; Cousins, 1972) and mother-to-child (vertical) transmission of yaws do occur, though rarely (Roman and Roman, 1986; Giacani and Lukehart, 2014).

Yaws and endemic syphilis are prevalent diseases of children in hot humid and hot dry remote areas of developing countries with over-crowded poor communities, scarce water supply, unhygienic conditions and poor sanitation (Widy-Wirsky, 1985; Engelkens *et al.*, 1991b; Mitja *et a.l,* 2013a). Pinta, which is endemic to Mexico, Central and South America, is also a mild treponemal disease characterized by formation of red papules in the skin. Detailed clinical manifestations involving stages of *T. pallidum* infection in humans namely primary, secondary and tertiary are reviewed in details elsewhere (Mitja *et al.,* 2013a; Giacini and Lukeheart, 2014; Marks *et al.,* 2014).

Syphilis

Syphilis is a prevalent disease particularly in low- and middle-income countries with an estimated global burden of 36 million cases and 11 to 12 million new cases per year (WHO, 2001; 2011). Up to 90% of these cases are reported from the developing countries

(Stamm, 2010), and men having sex with men (MSM) have increasingly been affected in developed countries including United States, China, England, Canada and Australia (Botham *et al.,* 2013; Peterman and Weinstock, 2015; Burchell *et al.,* 2015; Chen *et al.,* 2016; Petersen *et al.,* 2016). In Sub-sahara Africa, the pooled prevalence of syphilis in pregnant women was estimated at 2.87% with the highest prevalence of 2.49% in South Africa, East Africa being at 2.17% and the least is 0.16% in West Africa (Hussein and Tadesse, 2019). In Tanzania, Manyahi and others (2011) reported overall syphilis prevalence among pregnant women was 2.5 % with significantly higher risk of infection among women attending semi-urban and rural clinics and those having 3–4, and 5 previous pregnancies ($p < 0.05$). In Rwanda, Mutagoma and others (2016) reported prevalence of syphilis of 0.9% in HIV infected people with the 25-49 age group, women with lower education and HIV infected people being more affected. Similar findings were obtained in Kenya, where HIV-infected people had higher prevalence of 6.4% compared to HIV-negative individuals, who had 1.6% (Otieno-Onyunya *et al*., 2011).

Mother-to-child (vertical) transmission of syphilis is on the rise with a worldwide estimate of 1.36 million infected pregnant women each year, of which over 500,000 ended up with adverse outcomes such as spontaneous abortion, stillbirth, premature delivery, neonatal death and manifestations of congenital syphilis (Newman *et al.,* 2013). In addition, neurosyphilis, which is central nervous system invasion by *T. pallidum* associated with syphilis, affects about 40% of early syphilis patients (Lukehart *et al.,* 1988; LaFond and Lukehart, 2006; Radolf *et al.,* 2014). Neurosyphilis and cardiovascular syphilis respectively cause psychiatric disorders and deaths of many people in pre-antibiotic era (Holmes *et al.,* 2007).

Syphilis has primary, secondary, latent and tertiary stages with different clinical manifestations though sometimes there is lack of clear demarcation between the stages. The incubation period varies from 3 to 90 days but there is significant variability in morphologic presentation of the lesions (Chapel, 1978; DiCarlo and Martin, 1997; Singh and Romanowski, 1999). In the primary stage, the classic syphilis is manifested by eruption of a single or sometimes multiple chancre(s) at the site of inoculation. A chancre is an indurated ulcer of about 0.3-3.0cm in size with a clean base, which is painless (Chapel, 1978; Singh and Romanowski, 1999). The most common sites that a chancre prefers in men include coronal sulcus, glans penis and anorectal areas (for homosexuals) but in women, the labia majora, labia minora, fourchette and perineum (Stokes *et al.*, 1944, cited by Singh and Romanowski, 1999; Chapel, 1978). Extragenital chancres are usually painful and infrequently observed on the fingers, border of the tongue, anus and mouth, especially the lips (Tucker and Mulherin, 1948; Mindel *et al*., 1986).

Secondary syphilis lesions may easily be confused with other dermatological diseases (Chapel, 1980; Mindel *et al*., 1986). Primary chancre(s) may still exist in one-third of patients or heal for about 8 weeks before any cutaneous signs re-appear. The copper-colored or “raw ham” skin lesions (rash) variably appear ranging from macular to maculopapular, follicular and, rarely, pustular (Stokes *et al.*, 1944, cited by Singh and Romanowski, 1999; Chapel, 1980). The lesions spread all over the body though the palms and soles are commonly involved with variable degrees of pruritus or without (Stokes *et al.*, 1944, cited by Singh and Romanowski, 1999; Chapel, 1980). If untreated, the lesions resolve over several weeks and heal with or without scarring or hyperpigmentation or hypopigmentation. Patchy alopecia with a “moth-eaten appearance” may also feature in up to 7% of patients (Chapel, 1980; Mindel, 1989). The skin lesions may associate with pharyngitis and tonsillitis that result into a symptomatic sore throat and mucus patch,

typical lesion of the mucus membranes involving the tongue, buccal mucosa, and lips may occur in 5 to 22% of patients (Chapel, 1980; Mindel, *et al*., 1989). The lesions are slightly raised with central erosion covered with a thin membrane, painless and variable sizes from 5 to 10 mm (Sing and Romanowski, 1999). Genital mucusa lesions are more common in women and range from macules to papules, ulcerations and condylomata (Stokes *et al.*, 1944, cited by Singh and Romanowski, 1999). The lesions last 2 to 3 weeks though may disappear within hours to days. Systemic symptoms include slight malaise, prostration, cachexia but headache, low-grade fever, meningitis and cranial nerve palsies infrequently occurred (Stokes *et al.*, 1944, cited by Singh and Romanowski, 1999). Gastrointestinal symptoms include anorexia, nausea and occasionally vomiting (Singh and Romanowski, 1999). Painless adenopathy most commonly involving the suboccipital, cervical (posterior cervical), posterior auricular and epitrochlear nodes have also been reported (Stokes *et al.*, 1944, cited by Singh and Romanowski, 1999).

The latent or asymptomatic stage of syphilis is the period between disappearance of secondary manifestations to development of tertiary manifestations or until when the therapeutic cure occurs. First relapses occur within 1 year while others occur within 2 years and the rest over 4 years after the initial infection (Gjestland, 1955). Disappearance of the lesions in less than 1 year signifies early latent syphilis while any time span beyond means late latent syphilis. Due to existence of 25% risk for early latent syphilis patients to relapse into secondary syphilis, these patients are considered to be infectious (Gjestland, 1955).

Tertiary syphilis mainly manifests in two forms that are cardiovascular syphilis and neurosyphilis, involving the heart and the brain, respectively. In the prepenicillin era, cardiovascular syphilis accounted for 10 to 15% of all clinical cardiovascular disease

(Mindel *et al*., 1989). Neurosyphilis is characterized by neurological signs and associated complications. Five major categories with frequently overlaps are known to exist; these are asymptomatic, meningeal, meningovascular, parenchymatous and gummatous. Patients with concurrent HIV infection experience unusual presentations and rapid progression of syphilis (Musher, 1990).

Yaws

Scanty information is available on current prevalence of yaws due to poor disease surveillance (Harper and Knauf, 2013). By 2008, yaws was reported by 14 countries in the tropical belt of Africa, South East Asia and the Pacific that had this disease since 1950s (WHO, 1953; Agadzi *et al.,* 1983; Fegan *et al.,* 1990; Tharmaphornpilas *et al.,* 1994; Manning and Ogle, 2002; Asiedu, 2008; Fegan *et al.,* 2010; WHO, 2012). In early 1953, there were between 50 and 150 million active cases of yaws in 90 countries (Hackett, 1953; WHO, 2012) and much more people are at risk of contracting the disease in the actively infected areas. Mitja and others (2015) estimated that over 89 million people reside in yaws-endemic districts in 2012. More recent cases of yaws were reported from low to middle-income countries namely the Central African Republic (WHO, 2012), Democratic Republic of Congo, Vanuatu, East Timor and Papua New Guinea (Gersti *et al.,* 2009; Fegan *et al.*, 2010; Satter and Tokarz, 2010; Mitja *et al.*, 2011).

Briefly, the incubation period of yaws 9 to 90 days following infective exposure (Perine *et al.*, 1984). After this, the primary stage occurs and is featured by formation of a ‘mother yaw’ (‘buba madre’ or ‘mamapian’), mostly on the lower extremities or buttock, arm, face or hand (Perine *et al*., 1984; Antal *et al.,* 2002; Kartz *et l.,* 2018). This lesion is a non-painful, though may be pruritic, solitary papule of 2-5 cm-diameter with raised dark margins and erythematous moist center (raspberry-like). The papule can extend peripherally to form a papilloma or coalesce with other surrounding papules (Perine *et al*.,

1984; Schgal, 1990; Antal *et al.*, 2002). Sometimes, a group of small dry papules and rarely lack of observable primary lesions may replace the typical primary lesions with or without regional lymphadenopathy and arthralgia (Powell, 1923; Engelkens *et al*., 1991b; Koff and Rosen, 1993). Mostly, the primary lesions heal spontaneously before the secondary clinical signs set in about 1 to 2 years post infection (Giacini and Lukeheart, 2014).

Secondary smaller ‘mother yaw’-like papules or scaly irregular shaped maculae may also expand and ulcerate to release a highly infectious fluid (Giacini and Lukehart, 2014), formation of hard mass, *condylomata lata* in soft parts with moist folds including the axilla and groin, measles-like eruptions and hyperkeratotic plaques on palms and soles are common (Gip, 1989; Koff and Rosen, 1993). More ulcers, papillomata and/or papules, squamous macules as well as palmar and plantar lesions develop (Giacani and Luke hart, 2004; Katz et al., 2018). Rarely, periostitis of the long bones (saber shin) and fingers (polydactylitis) may develop in the secondary stage and, later on, non-infectious manifestations set in and result into destructive lesions of skin and bone in up to 10–20% of untreated individuals (Katz et al., 2018). At these early stages, fever, malaise, periostitis and osteitis of tibia, fibula, and forearm and proximal phalanges of fingers and toes with bone pain and digital swelling are common signs (Vanthournout *et al.*, 1991). The secondary lesions may also spontaneously heal in weeks or months and, if untreated, lead into a life-long latent stage in which with positive serological reactions are maintained with or without 5 to 10-year recurrences (Perine *et al*., 1984: Engelkens *et al.*, 1991b; Giacini and Lukehart, 2014).

About 10% of untreated cases progress into the tertiary stage, featured with formation of subcutaneous gummatous nodules, chronic periostitis with bone-destructing pathological changes. Thes include: apparent bowing of the tibia, saddle nose and perforation or

collapse of the palate and nasal septum as well as bilateral hypertrophic periostitis of the paranasal maxilla and nasal bridge (Martinez and Mouney, 1982; Whittet *et al.*, 1988; Engelkens *et al*., 1991b; Mafart, 2002; Giacini and Lukehart, 2014).

Bejel

Worldwide, reports of endemic syphilis emanate from 22 countries that are found in the South and Sahel region of Africa and the Middle East (Giacani and Lukehart, 2014; Marks *et al*., 2014). In addition, several imported cases were reported in France (Vabres *et al*., 1999; Grange *et al*., 2016) and Canada, which originated from Pakistan, Mali, and the Republic of Senegal (Fanella *et al.*, 2012). With exception of very few case reports, there are almost no recent systematic data on bejel and pinta prevalences in many countries where these diseases attracted public health significance (Mark *et al*., 2014).

Bejel's primary stage lesion is often unobserved though appears as a small and painless mucous papule or ulcer that develops in the oral cavity or nasopharynx, nipple of a nursing woman and in the genitals of adults (Giacani and Lukehart, 2004). Secondary lesions are very similar and highly comparable to lesions produced in venereal syphilis that is mucous patches on the mucous membranes of the mouth, tonsils, tongue, lips and nasopharynx (Giancani and Lukehart, 2004). Other common signs include split papules at the labial commissures, which is similar to yaws, nonitchy skin eruptions, generalized swelling of the lymph nodes and inflammation of the laryngitis (Pace and Csonka, 1988). Secondary skin lesions are comparable to those observed in yaws and syphilis. Maculopapular or papulosquamous lesions and nonitchy generalized papular rash may feature in a minority of patients (Pace and Csonka, 1988). As in yaws, osteitis and periostitis of the long bones and hands may occur and cause nocturnal bone pain (Giacani and Lukehart, 2004). Secondary manifestations heal in 6 to 9 months, and the disease enters latency (Sehgal,

1990). The tertiary stage with very similar characteristics as those for yaws (gummatous lesions of the skin, mucosa and bone that may progress to destructive ulcers), may show up in 6 months to several years (Giacani and Lukehart, 2004). Skin lesions resolve in time and depigmented scars become surrounded by hyperpigmentation, destructive lesions of the palate and nasal septum are just like those in yaws and less severe bone changes (i.e., saber tibia) are common. Neuro- logical and cardiac involvement and congenital transmission are rarely reported (Giacani and Lukehart, 2004)

Pinta

As compared to other diseases caused by pathogenic treponemes, information on the epidemiology of pinta is very scanty. This disease is endemic to Central and South America with most of the cases reported in Colombia and Mexico (Wilcox *et al*., 1974; Perine *et al*., 1984), and a few cases of pinta were suspected in Cuba (Sáenz *et al*., 1940). Before 1950, there were about one million cases (Perine *et al*., 1984) but the number of cases drastically dropped following mass treatment campaigns and improved hygiene although no accurate estimate of its current prevalence is available (Fohn *et al*., 1987).

Young adults of up to 15 years of age are affected with chronic skin lesions are considered to be the disease's main reservoir (Marquez *et al.,* 1955). The primary lesion starts as a papule or an erythematosquamous plaque after the incubation period of about 1 week to 2 months with or without satellite lesions. The papules increase in size and coalesce to form patches with a pale center (Edmundson *et al.,* 1967). After months, many hypochromic patches or light-blue/grayish pigmentation forms with more marked color at the center of the lesions and the initial lesions may either heal, leaving a slightly pigmented or hypochromic area, or persist for years and become indistinguishable from the secondary lesions (Edmundson *et al.,* 1967). Regional generalized swelling of the lymph nodes is

eminent at this stage so as dissemination of small secondary lesions (pintids) as scaly papules that enlarge and coalesce in plaques and become hypo- or hyperchromic (Edmundson *et al.*, 1967).

1.5.2.2 Non-human primate infection

Infectious treponemes

Simian treponematosis was first reported in Guinea baboons in West Africa in the 1960s (Fribourg-Blanc *et al.*, 1963; Fribourg-Blanc and Mollaret, 1969). The responsible bacterium was only referred to as *T. pallidum* Fribourg-Blanc (F-B). This bacterium has recently been further characterized and found to be genetically similar to the causative agent for human yaws, *T. pallidum* subspecies *pertenue* (Zobanikova *et al*., 2013). More studies that genetically characterized *T. pallidum* isolates from free-ranging NHPs in protected areas of Tanzania and elsewhere in Africa affirmed this observation (Knauf, 2011; Happer *et al*., 2012; Knauf *et al*., 2012, 2017; Chuma *et al*., 2018). This is compelling evidence that at least African NHPs in their natural settings are infected with *T. pallidum* subspecies *pertenue* although isolates are genetically diverse. This is an indication that these bacteria have co-existed with and well adapted to their NHP hosts for several decades.

To date, little is known on infection of NHPs with *Treponema* subspecies and strains other than *T. pallidum* subspecies *pertenue* and *T. pallidum* Fribourg-Blanc, the first simian *T. pallidum* isolate from West Africa in 1966. It should be noted that the latter bacterium is closely related genetically to *T. pallidum* subspecies *pertenue* (Zobanikova *et al*., 2013). Earlier studies did experimental infection of *T. pallidum* isolates from humans that are responsible for syphilis, yaws and pinta into NHP models such as chimpanzees (Metchnikoff and Roux, 1903), monkey (Ashbury and Craig, 1907; Nichols, 1910) and

rabbits (Nicols, 1910). Chimpanzees can experimentally be infected with pinta (*T. carateum*) and develop lesions that are very similar to those in humans (Varela, 1969; Kuhn III *et al*, 1970; Chandler Jr. *et al*., 1972) though the affected skin ends up with hyperpigmentation or normal pigmentation instead of hypopigmentation at final stages (Smajs *et al*., 2018). Although details on zoonotic nature of *T. pallidum* infecting humans and NHPs are currently missing, humans and NHPs share a great deal of other pathogens like arthropod borne viruses such as dengue fever, yellow fever, Zika, chikungunya and West Nile viruses with more or less similar clinical manifestations (Weaver, 2013). The more humans and NHPs live in close proximity to one another with direct and indirect interactions, the higher the chances of zoonotic transmission of the shared pathogens between them. Therefore, investing efforts and resources in understanding various pathogens and diseases these pathogens cause in humans and NHPs as well as their epidemiology is strongly justifiable. The final goal should always be to safeguard human and animal health while conserving biodiversity with as minimal negative impacts as possible to the species concerned and their natural environments.

Geographical distribution

Non-human primates (NHPs) in the tropical belt of Africa have been infected by pathogenic *T. pallidum* since 1960s (Fribourg-Blanc and Mollaret, 1963; 1969). Among the African countries where simian treponematoses have been reported include: Guinea Bissau, Senegal, Cameroon, Democratic Republic of Congo (DRC), Central African Republic, Tanzania, Ethiopia, Uganda and Kenya (Fribourg-Blanc *et al*., 1963; 1966; 1969; Kuhn, 1970; Baylet *et al*., 1971a; Baylet *et al*., 1971b; Levrero *et al*., 2007; Felsenfeld and Wolf, 1971; Knauf, 2011; Knauf *et al*., 2012, 2017; Harper *et al*., 2012; Chuma *et al*., 2018; Knauf, S., personal communication, 2017). More recent information

of infected NHPs (macaques) came from Asia, specifically Indonesia and Singapore (Klegarth *et al.*, 2017).

Infected NHP species

Treponema pallidum infection in several NHP species in their natural habitats in Africa or those translocated abroad but originating from different parts of Africa has been reported since 1960s - 1970s to date (Fribourg-Blanc *et al*., 1963; Fribourg-Blanc *et al*., 1966; Fribourg-Blanc and Mollaret, 1969; Kuhn, 1970; Felsenfeld and Wolf, 1971; Knauf, 2011; Harper *et al*., 2012; Knauf *et al.*, 2012, 2017; Chuma *et al*., 2018). The affected African NHP species as revealed by these studies include: patas monkeys (*Erythrocebus patas*), gorillas (*Gorilla gorilla*) and chimpanzees (*Pan troglodytes*), mangabeys (*Cercocebus* spp), green monkeys (*Chlorocebus sabeus*), colobus monkeys (*Colobus spp*.), Guinea baboons (*Papio papio*), olive baboons (*Papio anubis*), yellow baboons (*Papio cynocephalus*), vervet monkeys (*Chlorocebus pygerythrus*) and blue monkeys (*Cercopithecus mitis*).

Some NHPs, especially baboons in human yaws or syphilis-endemic areas, appear to have high seroprevalence of *T. pallidum* infection (Fribourg-Blanc *et al*., 1963, 1966; Fribourg-Blanc and Mollaret, 1969; Baylet *et al*., 1971a; Knauf *et al*., 2011; Harper *et al*., 2012; Knauf *et al*., 2015a, 2017). These findings increase the likelihood of zoonotic transmission of pathogenic treponemes between humans and NHPs in localities within or close to wildlife-human interfaces with high interactions in Africa. Among others, concurrent infection of NHPs in yaws-endemic areas has nowadays been under scrutiny. In Asia, 11 of 734 macaques representing 13 species were recently screened for treponematoses and found positive by serological tests; however, DNA could not be successfully extracted for further characterization (Klegarth *et al.*, 2017). According to Klegarth and others (2017),

the positive macaques originated from Southern and Western Sulawesi provinces (n=8) and Bali (n=2) in Indonesia and Singapore (n=1).

Clinical signs

Highly variable clinical manifestations have been described in NHPs ranging from lack of any lesions (asymptomatic infections) to mild keratotic lesions and ulcers around the muzzle, eyelids and armpits and severe anogenital and/ facial ulcerative lesions (Mollaret and Fribourg-Blanc, 1967; Baylet *et al*., 1971b; Wallis and Lee, 1999; Mlengeya, 2004; Harper *et al*., 2012; Knauf *et al*., 2011, 2012, 2015a; Chuma *et al*., 2018).

Majority of studied NHPs tested positive serologically but without notable clinical signs (Knauf *et al*, 2015a; Chuma *et. al.*, 2018). These include 90% (n=18/20) of Senegalese Guinea baboons (*Papio papio*) in West Africa (Knauf, 2015). Of these, 61% (n=94/154) of NHPs comprising of olive baboons (*Papio anubis*), yellow baboons (*Papio cynocephalus*) and vervet monkeys (*Chlorocebus pygerythrus*) in Tanzania (Chuma *et al*., 2018). These NHPs were probably in early sub-clinical phase of infection or were previously infected and had fully recovered by then. They could also be in the latent phase of the disease with possibilities to deevelop active disease in presence of stressors, suitable conditions and facilitating factors. Facial lesions were reported in 3.5% (n=3/86) of olive baboons of Tarangire and Serengeti National Parks in Tanzania with or without anogenital lesions and few observed in Lake Manyara National Park (Chuma *et al*., 2018).

Transmission

In natural settings, transmission within and across NHP species or between NHPs and humans as well as host specificity among treponemes are unclear (Mollaret and Fribourg-Blanc, 1967). Generally, it is known that *T. pallidum* has been transmitted by direct

contact with skin or mucous membrane of infected host with lesions. Indirect contact with the lesion exudate may also transmit the disease from one NHP to another. Transmission of *T. pallidum* possibly occurs during interactions among the NHPs in their day-to-day activities such as courtship, copulation, playing and fighting. In Tanzania, when simian treponematosis was first reported in Gombe National Park in 1989, only sexually active olive baboons were affected suggesting sexual transmission (Wallis and Lee, 1999). This is the reason for the use of the name 'sexually transmitted disease (STD) of baboons' since the disease was observed for the first time in Lake Manyara National Park in 1994 (Mlengeya, 2004). However, there is no other study on transmission among NHPs to affirm or disprove this suggestion.

A recent study by Knauf and others (2016) proposed possible involvement of flies after recovery of *T. pallidum* DNA was recovered from various fly species in baboon habitats. *T. pallidum* DNA from *Musca domestica* and *M. sorbens* flies that were in close proximity to infected olive baboons in their natural settings in both Lake Manyara and Tarangire National parks, Tanzania. *Stomoxys bengalensis, S. niger* and *S. sitiens* had *T. pallidum* DNA in Tarangire while *Chrysormia putoria* and unidentified fly species had the DNA in Lake Manyara (Knauf *et al*., 2016). Elsewhere in the world, involvement of flies as mechanical vectors was suggested previously after *M. sorbens* and *M. domestica* were demonstrated to transmit treponemes under experimental conditions (Thomson and Lamborn, 1934; Satchell and Harrison, 1953). If these widely spread flies can transmit *T. pallidum* subspecies *pertenue* among NHPs in the wild, and between NHPs and humans, the second WHO's treponematosis eradication campaigns is likely to be difficult.

Stages and fate of infection

Little is known on the epidemiology of *T. pallidum* subspecies *pertenue* infection in NHPs as compared to humans (yaws). It is also not known if *T. pallidum* infection in NHPs takes three stages namely primary, secondary and tertiary (Knauf *et al*., 2015a). However, different clinical signs have been observed in infected NHPs in the wild. At least the main known difference is that this bacterium causes mild to severe anogenital and/or orofacial ulcerations in NHPs while in humans and mostly children under 15 years, the yaws lesions are usually limited to the skin and, for chronic cases, cartilages and bones.

In Table 1, Knauf (2011) classified and scored the lesions of infected NHPs based on location in the body, severity, extent of spread and estimated levels of microbial contamination. Every NHP was given a specific identification number in the form of 02SNM2210217, whereas 02 stands for the running number of the NHP being handled, SN is the initials of the protected area (Serengeti), M or F for sex (Male and female, respectively), the sixth digit (e.g. 2, in this case) describes the location, extent and severity of the lesions while the last six digits (e.g. 210217), shows the date on which the animal was handled in DD/MM/YY (Day/Month/Year) format. The sixth digit scores the lesion as explained in Table 1 bellow:

Table 1: Scoring *T. pallidum*-caused ano-genital and/oro-facial skin lesions in NHPs

#	Female	Male	#
F1	Clinically non-affected		M1
F2	Clinically affected, initial stage, genital		M2

F3	Clinically affected, initial stage oral	M3
F4	Clinically affected, initial stage, oral/genital	M4
F5	Clinically affected, moderate stage, genital	M5
F6	Clinically affected, moderate stage oral	M6
F7	Clinically affected, moderate stage oral/genital	M7
F8	Clinically affected, severe necrosis, genital	M8
F9	Clinically affected, severe necrosis, oral	M9
F10	Clinically affected, severe necrosis, oral/genital	M10
F11	Clinically affected, oral and genital but differs in severity (please describe on the protocol)	M11
F12	Others	M12
F13	Others	M13

Source: Knauf, (2011): F stands for female and M for male.

There have been different opinions among scientists as to why these genetically closely related *T. pallidum* subspecies cause different clinical manifestations in humans and NHPs. Some people believe that *T. pallidum* subspecies have different predilection sites while others are of opinion that port of entry matters in determining location of the lesions in the host i.e. around the anogenital, orofacial, legs, ankles, arms, buttocks and elsewhere (Mulligan *et al.*, 2008; Lukehart and Giacani, 2014; Noda *et al*., 2018). Although there are some well-established facts supported with scientific data, it is not uncommon to find a couple of exceptions to the general trends. For example, neurological and cardiovascular invasion commonly associated with *T. pallidum* subspecies *pallidum* (syphilis) were also observed in yaws (Edington, 1954; Lawton-Smith *et al*., 1971; Roman and Roman, 1986). Intriguingly, genital lesions and sexual transmission that are usually observed in syphilis were also documented in bejel (Grin, 1952). Furthermore, *T. pallidum* subspecies *endemicum* was recently recovered from samples of several patients previously diagnosed with syphilis in Cuba (Noda *et al*., 2018). To sum it all, compelling evidence slowly accumulates in favour of the fact that pathogenic treponemes represent a genetic continuum with overlapping host ranges, clinical signs and modes of transmission being

more defined by the opportunity rather than the biology of the causative agent (Mulligan *et al*., 2008; Lukehart and Giacani, 2014; Noda *et al*., 2018).

Field observations in protected areas of Tanzania showed that anogenital ulcerations may be so severe in the NHPs to the extent of completely chopping off the penis or badly mutilate the vagina, anus and the surrounding tissues. In several events, the mutilations resulted into merging of the anal and vaginal openings to form fistulae. The orofacial lesions may also be severe, deep-seated and eating up the tissues to perforate the hard palate and expose the roots of incisor teeth and the tongue i.e joining together the nasal and oral cavities.

Other complications include: healing of the ulcerated tissues surrounding the male genitalia leaving abnormally smaller preputial opening thus compromising free extrusion of the penis and making the NHPs unable to perform intromission during mounting for copulation. Some affected NHPs were debilitated and experienced difficulties to move and, most likely, ended up falling easy preys to predators in the wild. In addition, more disabilities due to deformed bones have been observed in free ranging NHPs, especially the olive baboons in Lake Manyara National Park, Tanzania. Although no systematic data was collected, a good proportion of olive baboons had bone deformities in this Park as compared to their counterparts elsewhere in the country.

Diagnosis

Generally, treponemes have been diagnosed by dark field microscopy, serology (treponemal and non-treponemal tests), PCR and sequencing. The dark field microscopy is still regarded as the gold standard test for confirmation of treponematoses. However, microscopy and serology cannot differentiate the *T. pallidum* subspecies. Previously, PCR

could also detect pathogenic *T. pallidum* as a group but not discriminate between subspecies (Liu *et al*., 2001; Mitja` *et al*., 2011). With advancement of technology, nowadays PCR can segregate *T. pallidum* subspecies by amplifying specific gene loci.

Serology

Serological tests cannot distinguish one *T. pallidum* subspecies from the other (Centurion-Lara *et al*., 2006) but can detect the presence of these bacteria either directly (treponemal tests) or indirectly (non-treponemal tests). The former tests are non-specific and they include the venereal disease research laboratory (VDRL) and rapid plasma reagin (RPR). Basically, these tests detect antigen of cardiolipin, lecithin and cholesterol that patient-derived antibodies produce against the cell surface of *T. pallidum* to form visible flocculation (Marks *et al*., 2014) read microscopically (VDRL) or by the naked eye (RPR).

Treponemal tests include *T. pallidum* haemagglutination (TPHA), *T. pallidum* particle agglutination (TPPA) and Fluorescent Treponemal Antigen Absorption (FTA-Abs) tests. These are more specific in the sense that they detect antibodies even years post treatment (Naidu *et al*., 2012; Marks *et al*., 2014). The current clinical diagnosis of treponematoses, including yaws and bejel, mainly base on interpretation of clinical signs, serological results, geographical distribution and anamnestic data (Mitja *et al*., 2013b: Noda *et al*., 2018).

Rapid point-of-care treponemal tests in the form of immunochromatographic strips that do not require refrigeration are recently available (Kleutsch *et al*., 2009). Other tests have already been validated for use in NHPs, specifically for the olive baboons (Knauf *et al*., 2015b). However, the main disadvantage of these tests is that they cannot differentiate between active and treated infection (Mitja *et al*., 2013b). If diagnosis is based on the

treponemal tests alone, unnecessary treatments may be instituted raising probability of selecting for antibiotic-resistant pathogens (Mitja *et al*., 2013b).

Molecular detection

Studies applying PCR to detect treponematoses and whole genome fingerprinting techniques enabled identification of genetic signatures that can differentiate the existing treponemal strains (Mitja *et al*., 2013b). Pathogenic *T. pallidum* have generally been diagnosed by PCR techniques but without differentiating between subspecies and strains (Mitja *et al*., 2011). For example, Liu and others (2001) developed a PCR to detect pathogenic *T. pallidum* from several other multiple pathogens that cause similar lesions by targeting the deoxyribonucleic acid (DNA) Polymerase I (polA) gene. PCR assays and DNA sequencing-based techniques are more robust in differentiating pathogenic *T. pallidum* subspecies (Pillay *et al*., 2011; Flasarová *et al*., 2012; Chi *et al*., 2015).

PCR and sequencing of the *tpr II* followed by differentiation of subspecies basing on the presence of BsrDI restriction sites in *tprI* and *tprC* genes; segregate TEN from TPE (Centurion-Lara *et al*., 2006; Fanella *et al*., 2012). As compared to serological tests, PCR and DNA sequencing techniques are, however, more expensive and their use for routine diagnostic purposes is limited to fewer advanced laboratories in developed countries (Noda *et al*., 2018). In addition, recombination of genetic materials between two or more *T. pallidum* subspecies and/ strains do occur and may complicate the situation as it was for TPA and TEN (Mikalová *et al*., 2017). To avoid ambiguities and associated confusion that may arise as the case was with the classification of the human *T. pallidum* isolate Paris 11g/j (Grange *et al*. 2013; Mikalova *et al.*, 2014), sequencing of multiple treponemal loci is highly recommended in the classification of pathogenic *T. pallidum* into their appropriate subspecies (Nota *et al*., 2018). While targeting multiple loci increases

diagnostic resolution on the one hand, it increases diagnostic and/ research costs on the other.

The best diagnostic techniques for practical use especially in the developing world, where higher disease burdens are common, should be fairly cost-effective. Several efforts have been made to address this challenge. More recently, Knauf and others (2018) developed a multiplex loop-mediated isothermal amplification PCR (LAMP) assay, which detects and discriminates between TPA, TPE and TEN. With analytical detection limit of as low as $1x10^2$ treponemes per reaction, the assay is sufficient for diagnosis of primary and secondary syphilis (Tipple *et al*., 2011). The same numbers are also expected for yaws (Knauf *et al.*, 2018). Though not yet subjected to clinical and/ field trials to evaluate sensitivity and specificity, the assay is potentially beneficial for practical use in diagnosis of both human and simian treponematoses in remote and resource-scarce areas in developing countries.

Phylogenetic relationship

Phylogenetic analysis is aimed at depicting evolutionary relationship among the living organisms and group together genetically similar or closely related organisms. In such analysis, similar microorganisms like *T. pallidum* (TPAs, TPEs or TENs) do cluster together or at a neighbourhood regardless of their hosts of origin. So far there is no genetic evidence of zoonotic transmission of *T. pallidum* reported between humans and NHPs in the wild or in captivity. However, spatial and temporal connectivity between multiple infected NHP species and humans and existence of possible transmission routes in between increase risks for transmission of Treponematoses and other diseases between humans and NHPs (Knauf *et al*., 2016, 2017; Klegarth *et al*., 2017). All simian TPE isolates from African NHPs are paraphyletic to their counterparts from humans (Knauf *et*

al., 2017; Smajs *et al*., 2018). On the other hand, generally all human TPA isolates cluster into two main clades, namely Nichols-like and SS14-like (Smajs *et al*., 2018).

Treatment

Single intramuscular dose of long acting penicillin (benzathine benzylpenicillin) is used to treat human yaws at 1.2 MU for patients above 10 years and 0.6 MU for the younger (Perine *et al*., 1984). The cure rates exceed 95% (Mitja *et al*., 2013a). Treponemes disappear 8 to 10 hours after treatment with penicillin, healing of the skin lesions occur within 2 to 4 weeks and bone changes are reversible, if treated early enough (Marks *et al*, 2014). For syphilis, larger doses are recommended as *T. pallidum* penetrates tissues that penicillin cannot (Perine *et al*., 1984). One oral dose of azithromycin (30 mg/kg; maximum 2g), is also as effective as intramuscular benzathine benzylpenicillin in the treatment, control and eradication of yaws (Mitja *et al*., 2013a). However, currently the main challenge facing azithromycin is development of macrolide resistance, which is modulated by point mutation at Ala2058 (Stamm and Bergen, 2000) or Ala2059 of the 23S ribosomal RNA gene (Matejková *et al*., 2009). So far, the macrolide resistance has been observed only with syphilis-causing agent (TPAs) and has neither been reported in TPEs from humans (Stamm and Bergen, 2000) nor Tanzanian NHPs in which none of these mutations was detected (Chuma *et al*., 2019).

In NHPs, no systematic information is available on treatment of *T. pallidum* infection. However, a heavy dose of penicillin has been used to treat infected olive baboons in Gombe National Park in Tanzania with fairly good outcomes (Collins *et al*., 2011). Intriguingly, despite these efforts in Gombe being in place for about 30 years now, the disease has not been eradicated and is still prevalent (Chuma *et al.*, 2018). Furthermore, field experience in Tanzania shows that infected olive baboons and vervet monkeys

respond well to treatment with heavy doses of antibiotics. The lesions nicely regressed and the NHPs did fully recover. However, it is difficult to prevent the recovered free-ranging NHPs in the wild from being re-infected.

Infection in other wild and domestic animals and symbiotic relationship

Some cultivable treponemes cause a number of diseases including chronic infection of the oral cavity (periodontitis) in humans and digital dermatitis in domestic animals namely sheep, goats and cattle (Choi *et al.*, 1997; Rocas *et al.*, 2003; Siqueira and Rocas, 2004; Sayers *et al*., 2009; Duncan *et al*., 2014; Crosby-Durrani *et al*., 2016). Treponemes are also responsible for infection in wild animals, specifically the North American elks (Clegg *et al*., 2015). *Treponema paraluisleporidarum* ecovar Cuniculus (TPeC), which was previously known as *paraluiscuniculi* and formerly *Spirochaeta paralues-cuniculi,* infects rabbits (*Oryctolagus cuniculi*) since 1920 (Jacobsthal, 1920; Lumeij *et al*., 2013). *Treponema paraluisleporidum* ecovar Lepus (TPeL) infects the European brown hares (*Lepus europaeus*) and mountain hares (*Lepus timidus*) (Mörner, 1999; Knauf *et al*., unpublished data). While TPeC can be sexually transmitted with lesions including erythema, edema, crusting ulcers in the anogential areas; TPeL causes proliferative crusty skin lesions around orofacial and anogenital areas though the infection is mostly inapparent (Smajs *et al*., 2018).

Despite causing diseases of economic importance, treponema species are abundantly found in gut microbiomes of humans and non-human primates (NHP) but their roles are still not known (Bittar *et al* 2014; Schnorr *et al*., 2014). Some treponemes, such as those found in termite guts, are suggested to play symbiotic roles in their hosts and are linked with H_2-CO_2 acetogenesis and nitrogen fixation releasiing carbon and energy or providing nitrogen for their hosts (Graber *et al*., 2004).

1.6 NHP viruses of human health relevance

In humans, viral diseases of NHP origin cause big economic losses, resulting from their direct and indirect costs afflicted to the economy. NHPs are reservoirs for a wide range of macro and micro parasites of humans including helminths, protozoa, bacteria and viruses (Locatelli and Peeters, 2000).

Emerging infectious viruses

Nearly 75% of emerging infectious diseases of humans are zoonotic and majority originate from wildlife (Woolhouse and Gowtage-Sequeria, 2005; Cleveland *et al*., 2007). Over half of shared emerging pathogens in humans are viruses and the majority originate from wild NHPs (Jones *et al.*, 2008). NHPs are the known origins of human immunodeficiency virus (HIV) type 1 and 2 and influenza virus infections to humans (Assiri *et al.*, 2013). The NHPs also play a role in transmission of arthropod borne viruses including dengue fever, yellow fever, Zika, Chikungunya, and West Nile viruses (Weaver, 2013). Simian Immunodeficiency Virus (SIV), human T-lymphotropic virus, cytomegalovirus, hepatitis A, coronaviruses and respiratory syncytial virus also infect NHPs (Holmes, 2001; Bergmann *et al.*, 2006; Nunn and Altizer, 2006; Leendertz *et al.*, 2009; Drewe *et al.*, 2012). Increasing rates of disease transmission between humans and wildlife threaten public health and biodiversity conservation (Daszak *et al*., 2000; Jones *et al*., 2008). The current globally escalating COVID 19 pandemic, which is caused by Severe Acute Respiratory Syndrome (SARS) Coronavirus 2, has affected many people. Several wild and domestic animal species, especially NHPs and cats (Felidae) are variably susceptible; however, roles in spreading and maintaining this pandemic are unknown so as their fate.

Estimated economic losses from emerging viral infections

Globally, the Word Bank estimated the direct costs of outbreaks approximately more than US $ 20 but the costs hikes to about US $ 200 billions when the indirect impacts to the economy are included (Wolfe *et al.,* 2007). HIV/AIDS infected 36.9 million people causing 25 million deaths (Hahn *et al.,* 2000) while Ebola virus infected 31,033 with 12,913 deaths, including suspects (WHO, 2019). Estimated economic losses from highly pathogenic avian influenza costed the livestock sector US $10 billion in East Asia (Morens *et al.,* 2004; Wolfe *et al.,* 2007). In Tanzania, scanty information is available on viruses of NHPs and human viruses of NHP origin as well as their associated economic losses.

Simian virus detection rationale

Potentially zoonotic viruses infect NHPs singly or concurrently with *T. pallidum* necessitate screening for viruses of human health relevance. Detection of these viruses will facilitate devising strategies for early warming and prevent their transmission between humans and NHPs; hence, avoiding associated big economic losses. Determining association between these viruses and *T. pallidum* infection in Tanzanian NHPs is also of interest. Among others, some immunosuppressive viruses like Simian Immunodeficiency Virus (SIV) or Betaretrovirus may weaken host's immunity and predispose them to infections by other pathogens (Watchmna and Mansfield, 2012). This increases susceptibility of the human and/animal hosts to various pathogens including zoonotic viruses.

Some DNA viruses such as members of the family of *Herpesviridae* and *Papillomaviridae* are known to cause sexually transmitted infections in NHPs (Ford *et al.,* 1998) with lesions similar to those caused by *T. pallidum*. For example, *Herpesvirus papio 2* (HVP-2) and *Herpesvirus hominin* 2 do cause genital ulcerations in baboons (Bigger and Martin,

2002; Ford *et al.,* 1998). Other pathogens causing *T. pallidum*-like lesions (ano-genital ulcerations) including *Chlamydia, Haemophylus ducreyi* and *Klebsiella granulomatis* were ruled out (Knauf, 2011). In investigating genital diseases as the case is with *T. pallidum* infection, which is thought to be a sexually-transmitted disease (STD) in NHPs, it is critical to as well study and rule out other pathogens causing similar lesions. When *T. pallidum* subspecies *pertenue* was identified as the causative agent of the genital disease in the olive baboons at Lake Manyara National Park, several other pathogens causing similar lesions were ruled out (Knauf, 2011; Knauf *et al.*, 2012).

Viruses, especially ribonucleic acid (RNA) viruses are very prone to genetic changes in presence of favourable conditions. Some animals are incriminated to facilitate pathogens in undergoing genetic changes (e.g. mutations, reassortments, etc), that are translated into synthesis of different chemical composition, structure, performance, behavior of the pathogens and diseases caused. For example, pigs play important role in the reassortment of Avian Influenza virus to produce different haemaglutinin-nuraminidase (H-N) combinations with varying infectivity, severity and fate of diseases they cause in human and animal populations.

1.7 Organization of the Thesis

This PhD Thesis is organized in a way that Chapter One contains the introduction, problem statement and justification, overall objective, specific objectives, study design and sample size estimation. The Chapter also contains literature review on Treponemes and *T. pallidum* infection in humans, NHPs as well as domestic and wild animals. The Manuscript I, Paper I, Paper II and Manuscript II form Chapters Two, Three, Four and

Five, respectively. The Manuscript I (accepted by Springer Nature; Appendix 4) is a review of spirochetes with data from various previous studies and the current study. The results related to each specific objective of this thesis are presented and discussed in Journal Paper I (published by the *Emerging Infectious Diseases* journal), Journal Paper II (published by the *Scientific Reports* journal) and Manuscript II (to be submitted to the *Journal of Medical Primatology*). Chapter Six draws overall conclusions and puts forward recommendations emanating from this study. Attachments, appendices and references for the respective Journal Papers and the Manuscripts are attached at the end of the Papers or Manuscripts concerned. The thesis ends with the reference list for Chapters One and Six, Appendix 3 (the data collection Form used during chemical immobilizations of the NHPs) and Appendix 4, the Springer Nature's acceptance letter for the Manuscript II.

CHAPTER TWO

2.0 PATHOGENIC SPIROCHETES IN MONKEYS: STEALTHY PATHOGENS OF GLOBAL IMPORTANCE

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Abstract

Spirochetes are helical-shaped Gram-negative bacteria that are important for the health of both non-human primates (NHPs) and humans. However, little is known about the spirochetes that naturally infect NHPs. Lyme disease and relapsing fever are caused by bacteria of the genus *Borrelia*, obligate parasites transmitted by arthropod vectors. Due to the close phylogenetic relationship of humans and NHPs and the importance of *Borrelia* infections in humans, translational NHP models have been developed. Leptospirosis, caused by different pathogenic bacteria of the genus *Leptospira*, affects both humans and NHPs. Naturally acquired and clinically apparent leptospirosis is rare in NHPs. However, clinically healthy animals test positive for antibodies against the spirochete, indicating that NHPs might function as a disease reservoir for humans. Syphilis, yaws, and bejel represent infections caused by bacteria of the genus *Treponema*. Naturally occurring *Treponema* infections in NHPs, as well as the continual use of NHPs as experimental models for human treponematoses have been documented. With the objective of widening our understanding of these important pathogens, this chapter reviews and discusses three groups of spirochetes that cause considerable diseases in NHPs in the context of naturally and artificially acquired infection: *Borrelia*, *Leptospira*, and *Treponema*. Essential is the

One Health concept that addresses the connection and spread of diseases between humans and NHPs.

2.1 Introduction to Spirochetes

The order Spirochetales includes non-pathogenic and pathogenic bacteria (Paster, 2010). Some of these bacteria are free-living saprophytes, whereas others have kept their ancestral ability to survive in the environment, but have developed the ability to infect a broad range of animals and humans. On the other extreme, the group of spirochetes includes bacteria that are so specialized in their biology that they are unable to survive in the environment, as is the case for the syphilis-causing bacterium *Treponema pallidum* (*TP*). Spirochetes are an ancient and deeply branching phylum of Gram-negative bacteria and one of the few bacterial orders where phylogeny mostly reflects the organisms' cell morphology (Paster *et al.*, 1984; Caro-Quintero *et al.*, 2012). Almost all members of the phylum are helical-shaped and possess periplasmatic flagella (Paster and Dewhirst, 2000; Charon and Goldstein, 2002), which mediate motility. Taxonomically, the phylum *Spirochaetes* consists of a single class, *Spirochaetia*, which contains spirochetes in one order, *Spirochaetales*. Subsequently, the order *Spirochaetales* comprises the four families *Brachyspiraceae*, *Brevinemataceae*, *Leptospiraceae*, and *Spirochaetaceae*. The family *Brachyspiraceae* includes the genus *Brachyspira* and the family *Brevinemataceae* includes the genus *Brevinema*. *Leptospiraceae* includes the genera *Leptonema* and *Leptospira*, and the family *Spirochaetaceae* includes the genera *Spirochaeta*, *Borrelia*, *Cristispira*, and *Treponema* (Paster, 2015). Fig. 2 illustrates the phylogenetic relationship of selected spirochetes, including important pathogens for NHPs. The construction is based on GenBank published sequence data of the 16S rRNA gene.

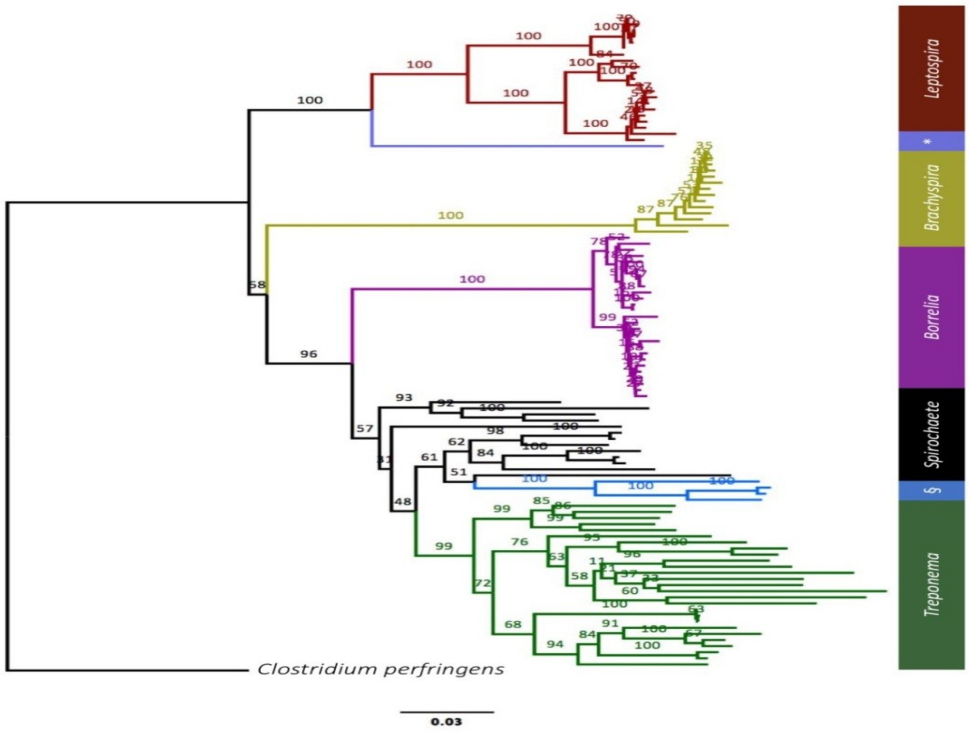

Figure 2. Bio-Neighbor-Joining consensus tree of selected spirochetes

The tree is based on the V2-V8 region of the 16Sr RNA gene with 1101 sites. The Jukes-Cantor substitution model was chosen and 1,000 bootstrap replicates were performed. Bootstrap values are displayed at respective branches. The bar refers to substitutions per site. *Clostridium perfringens* is used as an outgroup. **Leptonema illini*, §*Sphaerochaeta*.

Historically, little is known about the spirochetes that infect non-human primates (NHPs), although information has begun to accumulate using modern genetics. Three groups of spirochetes, namely *Borrelia*, *Leptospira*, and *Treponema*, are important for human health. However, not all of these pathogens naturally infect NHPs. While *Borrelia* for example is accountable for a major disease complex for human health, causing Lyme disease and relapsing fever, bacteria of this genus do not naturally infect monkeys. The second important disease complex is leptospirosis, which is caused by different pathogenic *Leptospira* organisms. In particular, rodents and bats have co-evolved with this pathogen

and function as a disease reservoir for the infection in both humans and NHPs. While infection in humans is of significant importance, natural infection in NHPs is mostly acquired during captivity and does not seem to play a major role in wild monkeys. The last complex contains diseases caused by *Treponema* and is of great importance for human and NHP health. Natural infection with *Treponema pallidum* is common in humans and in wild NHPs, which underlines the need for One Health investigation. In the following text, these three major pathogens, *Borrelia*, *Leptospira*, and *Treponema*, will be discussed in the context of naturally and artificially acquired infection in NHPs.

2.2 Borrelia

The genus *Borrelia* belongs to the ancient phylum of spirochete bacteria and includes important human and animal pathogens (Brisson *et al.*, 2012). The genome of the *Borreliae* is composed of a linear chromosome in conjunction with linear and circular plasmids (Wang and Schwartz, 2015). Most genes located on the chromosome are likewise found in other bacteria (Fraser *et al.*, 1997) whereas genes located on the plasmids are generally unique for the genus (Fraser *et al.*, 1997; Casjens *et al.*, 2000). Members of the *Borreliae* are obligate parasites transmitted by arthropod vectors (Cutler *et al.*, 2017). This distinguishes them from other spirochetes such as *Treponema* and *Leptospira*.

Based on DNA sequence analysis, two major phylogroups within the *Borrelia* genus can be distinguished (Wang and Schwartz, 2015). One group contains three pathogenic species (*B. afzelii*, *B. garinii* and *B. burgdorferi*) as well as seven minimally pathogenic to non-pathogenic *Borrelia* spp. (Wang and Schwartz, 2015). The most notable pathogen in this phylogroup is *Borrelia burgdorferi*, which causes Lyme borreliosis and was first isolated from the tick species *Ixodes scapularis* (Burgdorfer *et al.*, 1982). The phylogroup is generally named the *Borrelia burgdorferi* sensu lato (sl) complex and referred to as Lyme borreliosis spirochetes (LBS) (Ytrehus and Vikøren, 2012). A common feature of this

group is that transmission requires hard-bodied ixodic tick species (*Ixodes ricinus* complex).

The second phylogroup consists of a larger number of *Borrelia* spp. (more than 20) that are associated with relapsing fever. Members of this group are either louse borne (*Borrelia recurrentis*) or soft tick transmitted (Wang and Schwartz, 2015), with the exception of *B. theileri*, *B. miyamotoi* and *B. lonestari*, which are transmitted by hard ticks (Cutler *et al.*, 2017). The epidemiology of *Borrelia* infection is predictably associated with the geographic range of the respective arthropod-vector (Fig. 3). In addition, the distinct grooming behavior of wild NHPs makes it difficult for arthropod vectors to infect monkeys. However, the geographic ranges of hard-ticks and NHPs do not significantly overlap, and Lyme borreliosis is not a relevant disease in monkeys. Reports on natural infection are absent (Pritzker and Kessler, 2012). *B. harveyi*, however, has been considered to be naturally associated with a monkey reservoir (Ytrehus and Vikøren, 2012; Wang and Schwartz, 2015). Generally, rodents account for most of the known host and reservoir species, but there are also *Borrelia* species that infect birds and reptiles (Ytrehus and Vikøren, 2012).

In humans, infection with *B. burgdorferi* develops in three stages. After infection, a pathognomonic skin rash, the erythema migrans, develops in addition to fatigue or flu-like symptoms. The latter does not involve the respiratory tract. Left untreated, the disease enters its secondary stage within weeks where neurological signs such as meningopolyneuritis and myo-peri-pancarditis become present. The final stage occurs several months after infection and is associated with severe and painful polyarthritis of the major joints and chronic encephalomyelitis. Further details on clinical manifestations and definitions can be found elsewhere (Stanek *et al.*, 2012). Due to the importance of *Borrelia* infection in humans and the close phylogenetic relationship of humans and NHPs, translational NHP models have been developed (Pachner *et al.*, 1998; Embers *et*

al., 2012; Crossland *et al.*, 2017). While animal models in rats, mice, hamsters, guinea-pigs, gerbils, and rabbits develop arthritis only if an individual is immunocompromised (as reviewed in (Philipp and Johnson, 1994)), rhesus macaques (*Macaca mulatta*) when inoculated with *B. burgdorferi*, consistently develop the full range of human Lyme disease symptoms including neuroborreliosis (Roberts *et al.*, 1995; Pachner *et al.*, 2001; Embers *et al.*, 2012).

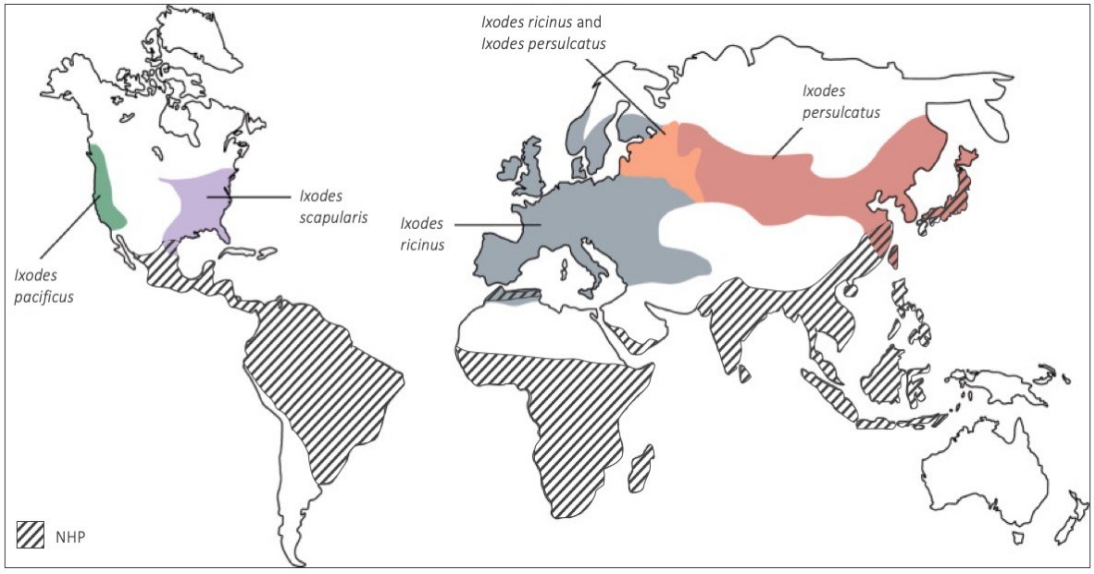

Figure 3. Geographic distribution of arthropod-vector for Lyme borreliosis and the geographic range of the wild non-human primates

The geographic ranges of Lyme borreliosis and NHP distribution have little overlap, which is predictive for the absence of naturally occurring Lyme disease in monkeys. Map source: The Lancet 2012 379, 461-473 (doi: 10.1016/S0140-6736(11)60103-7; copyright © 2012 Elsevier Ltd. Modification: overlay of NHP distribution).

2.3 Leptospira

Leptospira has been detected in almost all mammalian species which have been investigated (Adler *et al.*, 2011). Various wild and domesticated animals function as the disease reservoirs (Andersen-Ranberg *et al.*, 2016), yet rodents are the primary maintenance species for human infection (Ko *et al.*, 2009). The pathogen has a broad global distribution and can be found on all continents except Antarctica (Adler *et al.*, 2011). Despite its adaptability, *Leptospira* favors tropical conditions which are conducive to its transmission cycle (Bharti *et al.*, 2003). Based on 16S rRNA sequence data, three different clades can be distinguished (Lehmann *et al.*, 2014). *Leptospira* evolved from a free living non-infectious environmental organism (Lehmann *et al.*, 2014), which is reflected in the basal positioning of the non-pathogenic saprophyte containing clade (Fig. 4). The two pathogen containing groups include 14 species.

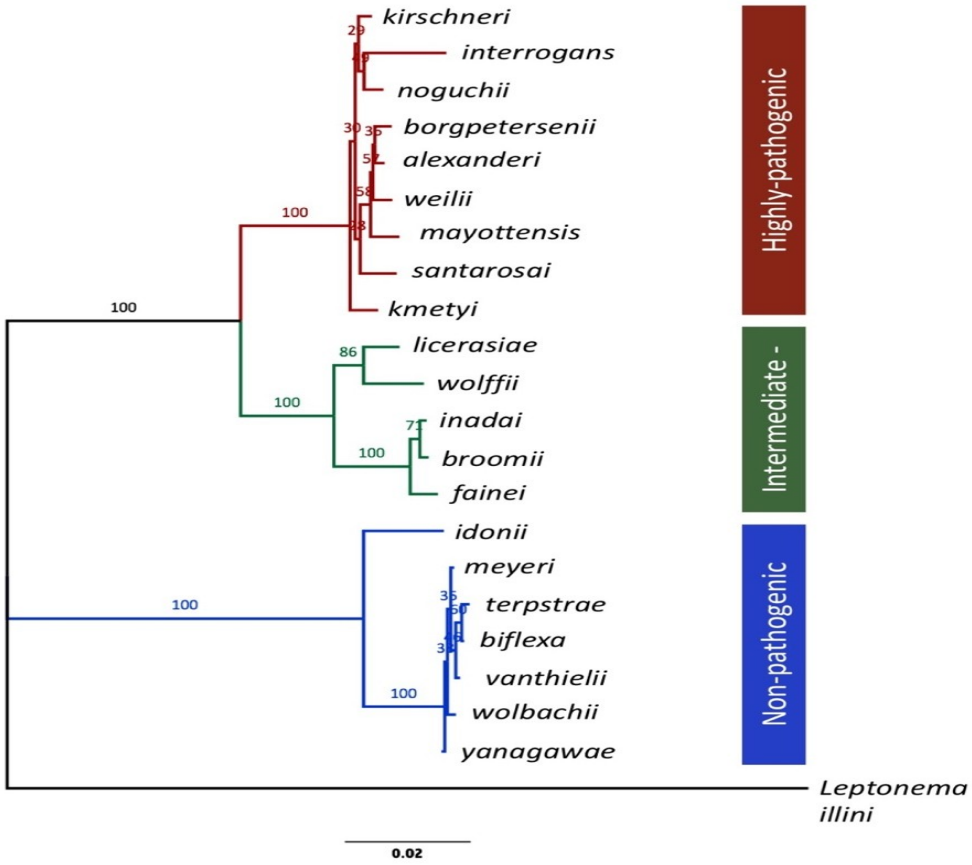

Figure 4. Bio-Neighbor-Joining consensus tree of selected *Leptospira* spp

The tree is based on the V2-V8 region of the 16Sr RNA gene with 1,189 sites. The Jukes-Cantor substitution model was chosen and 1,000 bootstrap replicates were performed. Bootstrap values are displayed at respective branches. The bar refers to substitutions per site. *Leptonema illini* is used as an outgroup. For the interested reader, a more detailed phylogeny can be found in Thibeaux and others (2018).

Leptospira species have the largest genome size among the spirochetes (>3.9-4.6 Mb), which is about four times the size of the *Treponema pallidum* genome of 1.1 Mb (Fraser et al., 1998; Picardeau, 2015). The *Leptospira* genome is circular and comprises at least two replicons (Zuerner, 1991). A third circular replicon (*p47*) has been identified in the non-pathogenic *L. biflexa*. Classification of *Leptospira* spp. into serovars and serogroups is based on their agglutinating antigenic composition, mediated by surface exposed lipopolysaccharide. The serovar classification is based on isolate-specific antigenic cross-matching; whereas serogroups are identified using microscopic agglutination tests (MATs). Pathogens of the *L. interrogans*-containing clade are subdivided into more than 250 serovars (sv), some of which are associated with renal carriage by a specific mammalian maintenance host species, while others may colonize a broader range of mammals (Lehmann *et al.*, 2014; Ellis, 2015). This association is not absolute, as a single animal species can carry different serovars in geographically distinct populations (Alexander *et al.*, 1963; Everard *et al.*, 1976, 1980; Tomich, 1979).

In contrast to the serovar classification, serogroups have no official taxonomic status (Balamurugan *et al.*, 2013). Within one serogroup as well as within one *Leptospira* sp. there can be several serovars. However, multi-locus sequence typing (MLST) has been shown to accurately classify *Leptospira* (Ahmed *et al.*, 2006), but whole genome sequencing is now considered the standard (Levett and Picardeau, 2018). However,

serotyping remains a key tool for epidemiological investigations (Cerqueira and Picardeau, 2009; Balamurugan *et al.*, 2013).

Pathogenic *Leptospira* species have co-evolved with their respective maintenance host, e.g., bats where the pathogens cause little to no clinical symptoms (Lei and Olival, 2014; Gomes-Solecki *et al.*, 2017). In the chronically infected maintenance host, the pathogen colonizes the proximal renal tubules from where it is shed to the environment in the urine (Ratet *et al.*, 2014; Gomes-Solecki *et al.*, 2017). Transmission requires continuous enzootic circulation of the pathogen among the maintenance host population (Ko *et al.*, 2009). Key to the transmission cycle of *Leptospira* is its ability to survive in the environment. Under ideal conditions, *L. interrogans* for example can survive for up to 28 days in fresh water (Casanovas-Massana *et al.*, 2018). In the laboratory, viable leptospires have been recovered after storage for several years. Susceptible hosts are infected when the highly motile bacterium penetrates abraded skin or mucous membranes. What follows is a rapid systemic infection which leads to the elimination of the bacterium from the blood stream and the chronic bacterial colonization of the proximal tubules of the kidney (Cinco, 2010; Ratet *et al.*, 2014).

In the susceptible non-maintenance host, tissue damage in multiple organs can be observed (Ko *et al.*, 2009). The initial hallmark of leptospirosis in humans is a non-specific febrile illness (McBride *et al.*, 2005). In 5-15% of all cases, leptospirosis can end in the most severe disease forms known as the hepato-renale syndrome (Weil's disease) and the severe pulmonary hemorrhage syndrome (SPHS). With case fatalities >10% in Weil's disease and >50% in SPHS, these diseases are a major health burden for humans (McBride *et al.*, 2005). Despite their enormous importance as human pathogens, and although NHPs and humans in the tropics often share the same contaminated habitats, little is reported about naturally acquired infections in NHPs.

Table 1 provides a summary of the reported cases of naturally acquired leptospirosis in NHPs. The early perception that naturally acquired and clinically apparent leptospirosis is rare in NHP is still valid today (Lapin, 1962; Simmons and Gibson, 2012). While New World monkey (NWM) species and Old World monkeys (OWM) can be experimentally infected with *Leptospira*, there is some indication that naturally acquired infection is only found in wild OWMs (Minette, 1966; Simmons and Gibson, 2012). This discrepancy could possibly be an effect of the arboreal lifestyle of most of the NWMs (Minette, 1966), as the contact with pathogenic *Leptospira* in the soil is minimized. This inference is further supported by a study in arboreal living Galagos (*Galago senegalensis*) in Africa, which tested negative for *Leptospira* antibodies (Minette, 1966). However, only small samples of Galagos were tested in this study and there are currently no published studies that tested wild NWMs. Additionally, there is some indication that wild-caught monkeys (NWMs) frequently acquired infection during their time in captivity (Minette, 1966).

According to Minette (1966), natural infection of Macaques (*Macaca* sp.), including a cynomologous monkey (*Macaca fascicularis*), Guinea baboon (*Papio papio*) and hamadryas baboons (*Papio hamadyras*), as well as chimpanzee (*Pan troglodytes*) were previously observed by Sanderson (1957). However, we were not able to confirm this information while reviewing the original work of Sanderson (1957).

Table 1. Summary of the reported cases of naturally acquired *Leptospira* infection in captive and wild NHPs

Geographic region of NHP species origin	Species common name	Scientific name	Number of animals diseased (total tested)	Captive (C) or wild (W)	Clinical symptoms (number)	Reference
South America	Common marmoset	*Callithrix jacchus*	16 (28)	C	NA	(Pinna *et al.*, 2012)
	Weid's marmoset	*Callithrix kuhlii*	2 (2)	C	Jaundice, anemia, renal failure, death (n=2)	(Baitchman *et al.*, 2006)
	Black-pencilled marmoset	*Callithrix pennicilata*	4 (8)	C	NA	(Pinna *et al.*, 2012)
	Cotton-top tamarin	*Saguinus oedipus*	1 (28)	C	Not described	(Minette, 1966)
	White-lipped tamarin	*Saguinus labiatus*	1 (1)	C	Lethargy and icterus, hemorrhage from the mouth, death	(Reid *et al.*, 1993)
	Capuchin monkey	*Cebus capuchinus*	5 (8)	C	NA	(Pinna *et al.*, 2012)
			unknown (15[$])	C	Not described	(Johnson and Morter, 1969)
	Common squirrel monkey	*Saimiri sciureus*	25	C	Mostly clinically healthy, 11 with Jaundice, hemorrhagic syndrome, deaths (n=10)	(Perolat *et al.*, 1992)
			unknown (15[$])	C	Not described	(Johnson and Morter, 1969)
			2 (15[$])	C	Depression, lethargy, respiratory distress, icterus, death (n=2)	(Johnson and Morter, 1969)
Asia	Macaques	*Macaca* sp. (species not determined)	4 (100)	W-caught	Not described	(Füzi and Csóka, 1963)
	Rhesus macaque	*Macaca mulatta*	3 (157) and 1 (47)	C and W	Not described	(Minette, 1966)
			1 (2)	W	Not described	(Hemme *et al.*, 2016)

			18 (59)	C	Not described	(Ibáñez-Contreras *et al.*, 2010)
	Barbary macaque	*Macaca sylvanus*	1 (2)	C	Pericarditis, death	(Urbain *et al.*, 1954)
			4 (104*)	C	NA	(Jaffe *et al.*, 2007)
			3 (26)	C	Jaundice, death (n=3)	(Shive *et al.*, 1969)
	Stump-tailed macaque	*Macaca arctoides*	2 (10)	C	Vomitus, depression, renal failure, death (n=1)	(Tschirch, 1989)
			3 (22)	C	Not described	(Minette, 1966)
	Bonnet macaque	*Macaca radiata*	9 (188)	W	Not described	(Minette, 1966)
	Hanuman langur	*Presbytis entellus*	8 (216)	W	Not described	(Minette, 1966)
	Lar gibbon	*Hylobates lar*	1 (13)	C	Not described	(Minette, 1966)
Africa	Baboon	*Papio* sp. (Species not determined)	170 (383)	C	Diarrhea, still births, some few deaths	(Fear *et al.*, 1968)
	Hamadryas baboon	*Papio hamadryas*	2 (104*)	C	NA	(Jaffe *et al.*, 2007)
	Guinea baboon	*Papio papio*	8 (31)	C	Not described	(Minette, 1966)
	Yellow baboon	*Papio cynocephalus*	11 (63)	C	Not described	(Minette, 1966)
	Patas monkey	*Erythrocebus patas*	2 (15[$])	C	Not described	(Johnson and Morter, 1969)
			6 (103)	C	Not described	(Minette, 1966)
			9 (22)	W	Not described	(Hemme *et al.*, 2016)
	Vervet monkey	*Chlorocebus sabeus*	139 (162)	C (n=81)/W (n=81)	NA	(Rajeev *et al.*, 2017)
	Grivet	*Cercopithecus aethiops*	6 (63) and 0 (8)	C	Not described	(Minette, 1966)
	Tana River crested mangabey	*Cercocebus galeritus*	1 (2)	C	Not described	(Minette, 1966)

49 (328)	C	Not described	(Minette, 1966)
24 (24)	W-caught	Jaundice, depression, hemorrhagic syndrome, death (n=23)	(Wilbert and Delorme, 1928, 1927)

*Total number of Barbary macaques plus hamadryas baboons; $The authors make no comment on the species composition. In total, 15 animals were included into the study. NA = not applicable (clinically healthy).

Naturally acquired infection summarized in Table 2, should be considered the best proxy for the pathogenesis of *Leptospira* in NHPs. Depression, respiratory distress, jaundice, and vomiting are among the most reported clinical manifestations. Death was not uncommon. However, many animals that tested positive for antibodies against the spirochete were described to be clinically healthy, indicating that NHP infection can progress sub-clinically.

Inoculation experiments with *Leptospira* are frequently described for diagnostic purposes in the pre-genomic era or for the development of translational animal models for human leptospirosis. The infectious doses or application routes used in these experiments do not necessarily reflect what can be expected under natural conditions. This means that the results can neither be directly translated into disease progression nor per se reflect the pathology in naturally infected NHPs. Nevertheless, these inoculation experiments, which have been conducted under standardized conditions, contribute to our understanding of leptospirosis in NHPs. While the clinical manifestations in naturally infected animals were more or less consistent across different primate species (Table 1), artificial infection results in some interspecies differences. Based on the historical data of the early 20th century, and although these data must be interpreted with caution in terms of accuracy of study design, infectious dose, and the description of pathological results, Asiatic macaques are reported to be less impacted by the pathogen with only febrile illness and nausea

(Huebner and Reiter, 1915, 1916; Uhlenhuth and Fromme, 1916; Pettit and Martin, 1920; Erber and Michaut, 1932; Babudieri, 1939; Badudieri and Bianchi, 1940).

The benign course of infection was also described for experiments where white-fronted capuchins (*Cebus albifrons*, (Noguchi et al., 1924)), baboons (*Papio* sp.; (Noguchi *et al.*, 1924)), and black spider-monkeys (*Ateles chamek*; (Noguchi *et al.*, 1924)) were artificially infected. In contrast to this, two marmoset species (*Saguinus oedipus* and *Saguinus geoffroyi*) and large-headed capuchins (*Sapajus macrocephalus*) could be infected with fatal consequences (Noguchi, 1919). There is however, a lack of consistency across the different studies and species. Stefanopoulo (1921) for example was able to induce a fulminant leptospirosis with fever and icterus over a course of 11 days in one toque macaque (*Macaca sinica*), which is an OWM species (Stefanopoulo, 1921). The animals were inoculated with a high dose of *Leptospira* from guinea pig tissue. The same was demonstrated in patas monkeys (*Erythrocebus patas*), which were also infected with *Leptospira* from tissue of diseased rats (Noc, 1920). A baboon (*Papio* sp.) that was infected by the same investigators, most probably using the same protocol and infectious dose, developed no abnormalities (Noc, 1920). The susceptibility of baboons to *Leptospira* infection was, however, demonstrated by intracranial injection, which led to meningitis and fever (Troisier, 1932). No icterus was present in these animals and both animals recovered.

In another study where three baboons were inoculated with a *Leptospira* isolate from a fatal chimpanzee infection, at least one baboon developed fatal infection (Wilbert and Delorme, 1927). Such inconsistencies across studies and species often make the interpretation of the inoculation experiments difficult; in addition, undiagnosed co-infections in the NHP models in these early experiments could have likely impacted the clinical outcome (Marshall *et al.*, 1980).

In recent years with the introduction of modern genetic techniques, NHP infection with *Leptospira* was no longer conducted for diagnostic purposes. Rather, monkeys are artificially infected with pathogenic *Leptospira* for the development of translational animal models for basic and applied research. These models have been shown to mimic the pathogenesis in human infection. The marmoset (*Callithrix jacchus*) as an established laboratory NHP has been successfully used to mimic the severe pulmonary form of leptospirosis (Pereira *et al.,* 2005).

2.4 Treponema

Treponemes are Gram-negative motile bacteria of 6 to 15 µm length and 0.1 to 0.2 µm diameters. Unlike most of the *Borrelia* and *Leptospira* species, treponemes contain a greater number of non-cultivable species (Šmajs *et al.*, 2018). The *Treponema* family furthermore contains pathogenic and non-pathogenic species, a classification which is based on the ability of the bacterium to cause disease in humans or animals. Among the most important diseases in humans is syphilis caused by the *Treponema pallidum* subspecies *pallidum*, yaws caused by the subspecies *pertenue* (TPE) and bejel caused by the subspecies *endemicum*. While syphilis is distributed globally, the other two subspecies are endemic diseases of which yaws is currently subject to global eradicating efforts (Asiedu *et al.*, 2014). *Treponema carateum,* a pathogen that was formerly classified as a *TP* subspecies, is the most benign of the endemic treponematoses and affects only the skin (Giacani and Lukehart, 2014). The geographic distribution of human endemic treponematoses is shown in Fig. 5. The phased disease progression with three subsequent stages is common for syphilis and the endemic treponematoses (Giacani and Lukehart, 2014; Radolf *et al.,* 2016). The initial lesion appears at the site where the bacterium enters the skin. The developing ulcers disappear spontaneously within a few weeks. In the meantime, the bacterium has disseminated in its host, causing variable systemic illness and

often a mucosal and skin rash. This is pathognomonic for the secondary stage of the disease, which again disappears spontaneously after some weeks or months. Tertiary stage infection is developed one to 20 years after acute infection and can lead to severe disabling conditions caused by cardiovascular and neurological sequelae and cartilage and bone deformation.

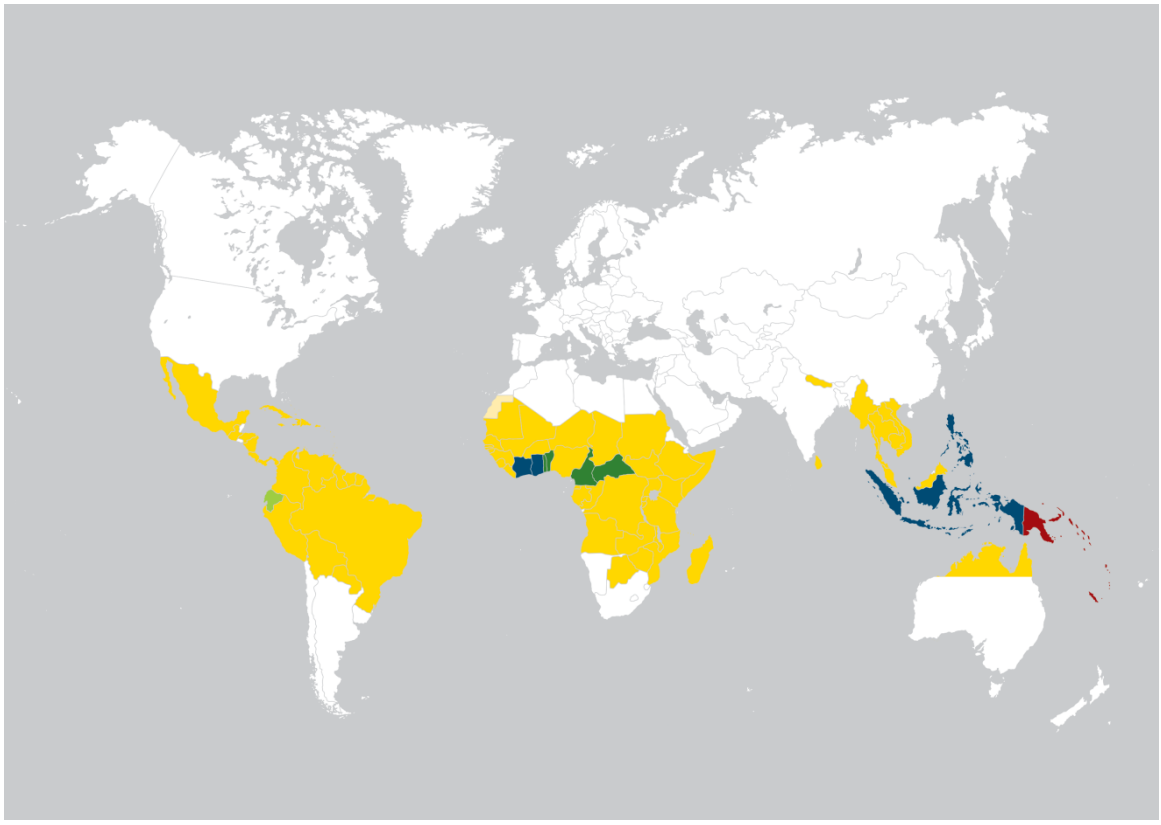

Figure 5. Global distribution of endemic treponematoses (Source: Knauf, 2018)

White = non-endemic, yellow = historically endemic (current status unknown), dark green = <1,000 cases, blue = 1,000-9,999 cases, red ≥ 10,000 cases in 2012. Copyright Bernhard-Nocht-Institute for Tropical Medicine (Knauf, 2018).

NHPs have been frequently used as a translational model for human treponematoses. Early experiments used NHPs including chimpanzees (Metchnikoff and Roux, 1903, 1904), toque monkeys (Castellani, 1907), cynomolgous monkeys (Ashbury and Craig, 1907), and rhesus macaques (Nichols, 1910). These early experiments spurred the continued use of NHPs as experimental animals for human treponematoses, though with limited success

(Turner and Hollander, 1957; Clark and Yobs, 1968; Elsas *et al.*, 1968; Sepetjian *et al.*, 1969, 1972; Marra *et al.*, 1998; Tansey *et al.*, 2017). Naturally occurring *Treponema* infection has been documented in NHPs (Hanson, 1970) and the pathogenic *TP* has been most frequently reported in wild NHPs in Africa (Knauf *et al.*, 2013). One of the first reports of an infected NHP came from a Guinea baboon (*Papio papio*) in West Africa in the 1960s (Fribourg-Blanc and Mollaret, 1969). The isolated bacterium was identified as *T. pallidum* strain Fribourg-Blanc and accounts for the first *TP* whole genome that was sequenced from an NHP isolate (Zobanikova *et al.*, 2013). The strain was genetically highly similar to human yaws-causing strains, which led to reclassification into subspecies *pertenue* (Zobanikova *et al.*, 2013). This finding supported the *pertenue*-like classification of earlier and current studies on *TP* in Tanzanian NHPs (Knauf *et al.*, 2011; Harper *et al.*, 2012; Chuma *et al.*, 2018) and remains supported by a growing number of published whole genome sequences of simian strains from West- and East Africa (Knauf *et al.*, 2018) (Fig. 6). Ongoing field work continues to demonstrate that a vast number of NHP host species can be infected (Table 2).

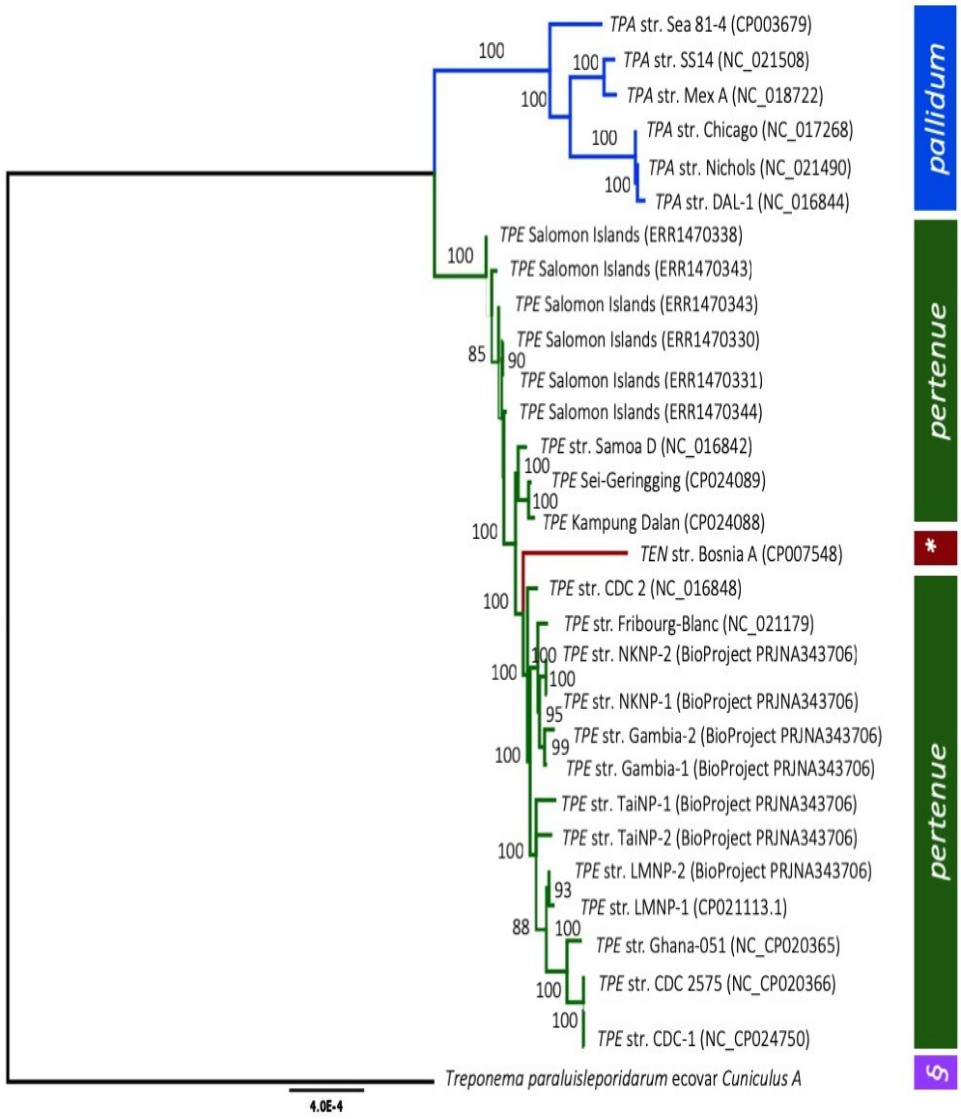

Figure 6. Bio-Neighbor-Joining consensus tree constructed from published *Treponema pallidum* whole genome sequences of human and NHP origin.

The Jukes-Cantor substitution model was chosen and 1,000 bootstrap replicates were performed. *Treponema paraluisleporidarum* ecovar *Cuniculus A* is used as an outgroup. Sequence accession numbers are provided in parentheses, TPA=*Treponema pallidum* susp. *pallidum*, *TPE = T. pallidum* subspecies *pertenue*, numbers indicate percent bootstrap support. * TEN = *T. pallidum* subspecies *endemicum* $outgroup rabbit syphilis.

Table 2. Summary of African NHP host species naturally infected with *TP*.

Species common name	**Scientific name**	**n animals diseased (total tested)**	**Reference**
Guinea baboon	*Papio papio*	64 (137)	(Baylet *et al.*, 1971b)
		18 (20)	Knauf *et al.* 2015
Olive baboon	*Papio anubis*	unknown	(Wallis and Lee, 1999)
		43 (57)	(Knauf *et al.*, 2011)
		86 (137)	(Chuma *et al.*, 2018)
		28 (52)	(Harper *et al.*, 2012)
Yellow baboon	*Papio cynocephalus*	33 (75)	(Chuma *et al.*, 2018)
		253 (835)	(Fribourg-Blanc and Mollaret, 1969)
Chlorocebus	*Chlorocebus spp.* (species not determined)	3 (15)	(Fribourg-Blanc and Mollaret, 1969)
		28 (45)	(Baylet *et al.*, 1971b)
Green monkey	*Chlorocebus sabaeus*	8 (8)	(Knauf *et al.*, 2018)
Vervet monkey	*Chlorocebus pygerythrus*	35 (45)	(Chuma *et al.*, 2018)
Chimpanzee	*Pan troglodytes*	3 (9)	(Fribourg-Blanc and Mollaret, 1969)
		unknown	(Kuhn, 1970)
Colobus monkey	*Colobus sp*. (species not determined)	1 (1)	(Fribourg-Blanc and Mollaret, 1969)
Sooty mangabey	*Cercocebus atys*	5 (5)	(Knauf *et al.*, 2018)
Patas monkey	*Erythrocebus patas*	7 (44)	(Fribourg-Blanc and Mollaret, 1969)
		1 (23)	(Felsenfeld and Wolf, 1971)
		2 (42)	(Baylet *et al.*, 1971)
Blue monkey	*Cercopithecus mitis*	2 (15)	(Chuma *et al.*, 2018)

*Infection was demonstrated by the presence of antibodies against *TP* and/ PCR.

A large number of naturally infected NHPs with antibodies against *TP* showed no signs of infection. This has been described in the majority of the published reports where wild NHPs were screened for infection (Baylet *et al.*, 1971b). Although the serologically positive animals displayed no skin lesions at the time they were sampled, it should be noted that a key feature of human treponematoses is the latent stage (Marks *et al.*, 2014). In this stage, infected individuals appear clinically healthy, but have both seroconverted and harbor the viable pathogen (Marks *et al.*, 2015). Assuming that the *TPE* strains of humans and NHPs share the same biology, clinically healthy but seroconverted NHPs are likely in the latent stage of the disease. In this case, all infected individuals should have had a primary skin ulcer following the initial infection, which spontaneously healed after

some weeks. Clinical manifestations of variable severity and extent have been described. These range from mild keratotic lesions and ulcers around the muzzle, eyelids and armpits in a baboon to more severe anogenital and facial ulcerative skin lesions in a number of different NHP species (Baylet *et al.*, 1971a; Wallis and Lee, 1999; Levrero *et al.*, 2007; Knauf *et al.*, 2011, 2018; Chuma *et al.*, 2018) (Fig. 7).

Figure 7. Genital skin lesions seen in a *Treponema pallidum pertenue* infected female olive baboon at Gombe National Park (left) and in a male vervet monkey at Mikumi National Park, Tanzania (Photo by Chuma, I. S. in 2016 & 2015, respectively)

It is currently unknown why simian *TPE* strains cause genital lesions, but it underlines the capability of *TP* subspecies to cause atypical lesions, something that has also been described for *endemicum* strains infecting humans (Grange *et al.*, 2016; Noda *et al.*, 2018). However, the genital ulcerative disease in Tanzanian NHPs suggests a sexual transmission mode, which is further supported by the predominant observation that sexually mature animals presented ulcerative skin lesions (Mlengeya, 2004; Wallis and Lee, 1999; Knauf *et al.*, 2011).

The epidemiological context of NHP-to-NHP or NHP-to-human infection and vice versa with *TPE* is unclear and subject to ongoing research (Fig. 8). The fact that NHP infecting *TPE* strains fall paraphyletic with human yaws-causing strains and the existence of *TPE* in

a number of different NHP species indicates that interspecies transmission must have occurred at least at some evolutionary stage. This however, does not provide evidence for ongoing transmission across different primate taxa including humans. The bacterium has a significant lack of metabolic activity, which limits its survival in the environment (Willcox and Guthe, 1966; Lafond and Lukehart, 2006). Therefore, infection must occur mainly through skin-to-skin or mucous membrane contacts (Richard *et al.*, 2017). A possible example for a conceivable interspecies transmission pathway was recently described through inverted intergeneric introgression in two different NHP species in Tanzania (Zinner *et al.*, 2018). Proving an epidemiological connection is complicated by the small number of high quality but also draft *TPE* genomes from African humans and NHPs. Suitable tools for genetic typing such as MLST are available (Pillay *et al.*, 1998; Marra *et al.*, 2010; Godornes *et al.*, 2017; Katz *et al.*, 2018), but require intensified sampling at locations where both NHPs and humans are infected.

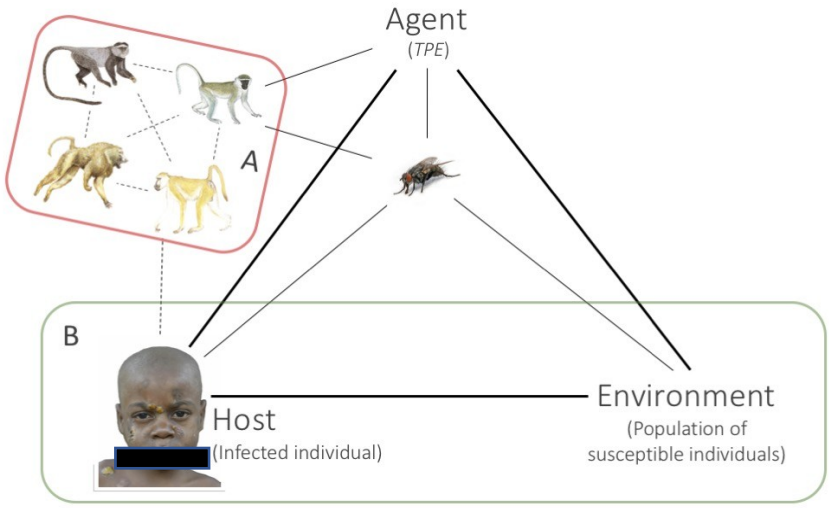

Figure 8. Triad of factors involved in the epidemiology of *Treponema pallidum* subspecies *pertenue* (bold lines)

In epidemiology the classical triad is made of the agent, the host, and the environment. (A) describes the possible reservoir system whereas (B) defines the target group. Thin lines describe published relationships, whereas dashed lines describe speculated connectivity. Connections are bidirectional. Figure sources: Human = https://www.spaceshipearth.org.uk/yaws.html (last accessed 05.06.2018; modified); NHP figures = (Kingdon, 2003) (modified); fly image source = https://www.oldskoolman.de/bilder/plog-content/images/freigestellte-bilder/natur-tiere/fliege-mit-ruessel.jpg (modified).

An alternative transmission pathway that would support interspecies transmission was described by Knauf and others (2016) who recovered *T. pallidum* DNA from captured wild flies trapped at close proximity to infected olive baboons in their natural habitat at Lake Manyara National Park and Tarangire National Park, Tanzania. This study was able to show that flies have regular contact with the pathogen, thereby providing a potential epidemiological link between humans and NHPs. However, these data did not demonstrate the viability of the pathogen, which is an important pre-requirement for a viable transmission route (Hallmaier-Wacker *et al.*, 2017). Under experimental conditions, fly transmission has been demonstrated (Thomson and Lamborn, 1934; Satchell and Harrison, 1953), although further genetic characterization of the treponemes which were used for these experiments was not conducted.

2.5 Other Treponematoses in NHPs

While chimpanzees experimentally infected with pinta developed human-like lesions (Varela, 1969; Kuhn, 1970; Chandler *et al.*, 1972), there are no reports of naturally occurring *T. carateum* infection in any of the NHP species from the New World, an area where pinta is circulating in human populations. Compared to *TP* infection, little is known about other pathogenic and non-pathogenic treponemes that infect NHPs. Some studies

report *Treponema* involvement in naturally occurring periodontitis in rhesus macaques (Colombo *et al.*, 2017) and experimentally induced periodontitis in cynomolgous monkeys (Sela *et al.*, 1987). Furthermore, an association with cardiac disease and treponemes of the gastrointestinal tract in gorillas has been reported (*Gorilla gorilla gorilla*) (Krynak *et al.*, 2017). Unfortunately, the majority of these studies were unable to specify the *Treponema* species. Apart from these descriptions of pathogenic treponemes, spirochetes, and in particular the genus *Treponema*, are found abundantly in gut microbiomes of humans and NHPs without knowledge of their pathogenicity (Bittar *et al.*, 2014; Schnorr *et al.*, 2014). With reference to the symbiont role that treponemes play in the termite gut system, it seems likely that treponemes in the gastrointestinal tract of herbivorous NHPs such as gorillas play a similar role (Breznak, 2002; Hicks *et al.*, 2018). A symbiotic role of gastrointestinal spirochetes in gorillas seems likely in light of the important role that this class of bacteria plays in the digestion of plant fibers (Schnorr *et al.*, 2014). In termites, treponemes contribute to carbon, nitrogen, and energy requirements, which help the host digest otherwise inaccessible plant materials (Warnecke *et al.*, 2007). Other studies report spirochetes and *Treponema* species as part of the vaginal microbiome in healthy baboons (Rivera *et al.*, 2011; Stumpf *et al.*, 2013; Yildirim *et al.*, 2014).

2.6 One Health

Although *Leptospira* and *Treponema* infections are frequently reported in NHPs, there is ongoing debate about the possible reservoir function that NHPs play for human infection. This is in particular the case for *TP*, where it is clear that African NHPs are infected with the yaws-causing subspecies *pertenue* (Knauf *et al.*, 2018). The first yaws eradication campaign, between 1952 and 1964, was successful in terms of reducing the global yaws prevalence by 95% (Asiedu *et al.*, 2014). However, decades later, yaws reemerged in West Africa, Southern Asia, and the Pacific region, which made it necessary to launch a second

eradication campaign (Asiedu *et al.*, 2014). While initial trials to treat yaws with a single dose of azithromycin were successful in Papua New Guinea, eradication efforts are impacted by a number of different variables such as a rapidly developing macrolide resistance (Mitjà *et al.*, 2018). The essential underlying question for eradication is whether or not there is ongoing interspecies transmission. Unfortunately, a number of unanswered questions prevent us from making a definite response (Hallmaier-Wacker *et al.*, 2017). In contrast to the possible transmission between NHPs and humans in Africa (Knauf *et al.*, 2013, 2018), it is unclear why infection with *TPE* is absent in wild but not pet macaques in Asia (Felsenfeld and Wolf, 1971; Klegarth *et al.*, 2017), despite the fact that human yaws is still endemic in large parts of the continent (Kazadi *et al.*, 2014).

If NHP-to-human infection exists, it is probably not the main driver for reemergence of yaws. However, eradiation of yaws requires an infinite zero-case scenario and even sporadic and seldomly occurring transmission between an existing non-human reservoir and humans would hinder yaws eradication. To achieve lasting eradication of yaws infection in humans and prepare for the worst-case scenario, it is therefore important to consider the role that NHP may play in the maintenance of yaws in non-human populations.

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CHAPTER THREE

RESEARCH

Widespread *Treponema pallidum* Infection in Nonhuman Primates, Tanzania

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We investigated *Treponema pallidum* infection in 8 nonhuman primate species (289 animals) in Tanzania during 2015–2017. We used a serologic treponemal test to detect antibodies against the bacterium. Infection was further confirmed from tissue samples of skin-ulcerated animals by 3 independent PCRs (*polA*, *tp47*, and *TP_0619*). Our findings indicate that *T. pallidum* infection is geographically widespread in Tanzania and occurs in several species (olive baboons, yellow baboons, vervet monkeys, and blue monkeys). We found the bacterium at 11 of 14 investigated geographic locations. Anogenital ulceration was the most common clinical manifestation; orofacial lesions also were observed. Molecular data show that nonhuman primates in Tanzania are most likely infected with *T. pallidum* subsp. *pertenue*–like strains, which could have implications for human yaws eradication.

The geographic distribution of infection with the bacterium *Treponema pallidum* in nonhuman primates (NHPs) in Africa has been reported to closely match the one seen in human yaws in Africa before the first yaws eradication campaign (*1*). Some Africa countries, such as Tanzania, have a history of human yaws but lack recent epidemiologic data that support elimination (*2*). At the same time, many of these countries report NHP infection with *T. pallidum* strains that are highly similar to the human yaws–causing *T. pallidum* subsp. *pertenue* (TPE) (*3*,*4*; S. Knauf et al., unpub. data, https://www.biorxiv.org/content/early/2017/05/10/135491) and thus make NHP infection an important issue for a One Health approach.

The first published report of *T. pallidum* infection in Tanzanian NHPs came from anogenital ulcerated olive baboons (*Papio anubis*) at Gombe National Park (GNP) in the late 1980s (*5*), followed by cases reported from olive baboons at Lake Manyara National Park (LMNP) (*3*,*6*,*7*) and Serengeti National Park (SNP) (*3*). Clinical manifestations of *T. pallidum* infection in NHPs ranged from asymptomatic to severe skin ulceration mainly affecting the face or genitalia (*8*). Although early serologic investigations conducted by Fribourg-Blanc in West Africa confirmed widespread infection in several NHP species (e.g., baboons [*Papio* sp.], guenons [*Cercopithecus* sp.], red colobus [*Piliocolobus badius*], and chimpanzees [*Pan trogoldytes*]) (*9*), the infection in Tanzania was exclusively reported from olive baboons in northern parts of the country. Despite the close genetic relationship to human yaws–causing TPE strains (*3*,*4*; S. Knauf et al., unpub. data, https://www.biorxiv.org/content/early/2017/05/10/135491), and in the absence of recent reports of human yaws in Tanzania (*10*), it is currently unclear whether NHP strains naturally infect humans.

As a starting point and basis for advanced epidemiologic investigations, our main objective was to investigate the geographic distribution and host species composition of *T. pallidum* infection in free-ranging NHPs in Tanzania. We hypothesized that, in Tanzania, A) NHPs other than olive baboons are infected with the *T. pallidum* bacterium and B) that infection is not restricted to northern parts of the country.

Materials and Methods

Study Design, Sampling Sites, and Animals

We applied a cross-sectional study design using semirandom selection of free-ranging NHPs in selected areas in

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[1]Deceased.

Tanzania. Selection of NHPs was biased toward animals with visible skin ulcers. Sampling took place at Arusha National Park (ANP), GNP, Katavi NP (KNP), LMNP, Mahale NP (MNP), Mikumi NP (MKNP), Ngorongoro Conservation Area (NCA), Ruaha NP (RNP), Selous Game Reserve (SGR), SNP, Tarangire NP (TNP), Udzungwa NP (UNP), and Issa Valley (Issa), as well as Jozani-Chwaka Bay NP–Masingini Forest (JCBNP) on Unguja Island, Zanzibar (Figure 1). We investigated the following species: olive baboon, yellow baboon (*Papio cynocephalus*), blue monkey (*Cercopithecus mitis*), red-tailed monkey (*Cercopithecus ascanius*), vervet monkey (*Chlorocebus pygerythrus*), Udzungwa red colobus (*Piliocolobus gordonorum*), Zanzibar red colobus (*Piliocolobus kirkii*), and Ugandan red colobus (*Piliocolobus tephrosceles*). Using FreeCalc (http://epitools.ausvet.com.au/content.php?page=FreeCalc2), and based on our previous study at LMNP (*6*) that showed a disease prevalence of 85%, we calculated a sample size of ≥4 (expected disease prevalence 85%) to 21 (expected disease prevalence 25%) per sample site as statistically sufficient to demonstrate freedom from *T. pallidum* infection using imperfect tests and allowing for small populations (online Technical Appendix 1, https://wwwnc.cdc.gov/EID/article/24/6/18-0037-Techapp1.pdf).

Anesthesia and Sampling

We studied the animals in accordance with applicable regulations and guidelines (online Technical Appendix 1). The sampling of blood and skin tissue followed a standardized protocol that we previously applied for baboons (*6*,*11*). In brief, the NHPs were chemically immobilized by remote distance injection of 10.0 mg ketamine/kg body mass (Kyron Laboratories, Johannesburg, South Africa) in combination with 0.2 mg/kg medetomidine (Domitor; Pfizer, Berlin, Germany). Anesthetics were intramuscularly injected using a cold-gas immobilization rifle (MOD JM; Dan-Inject ApS, Børkop,

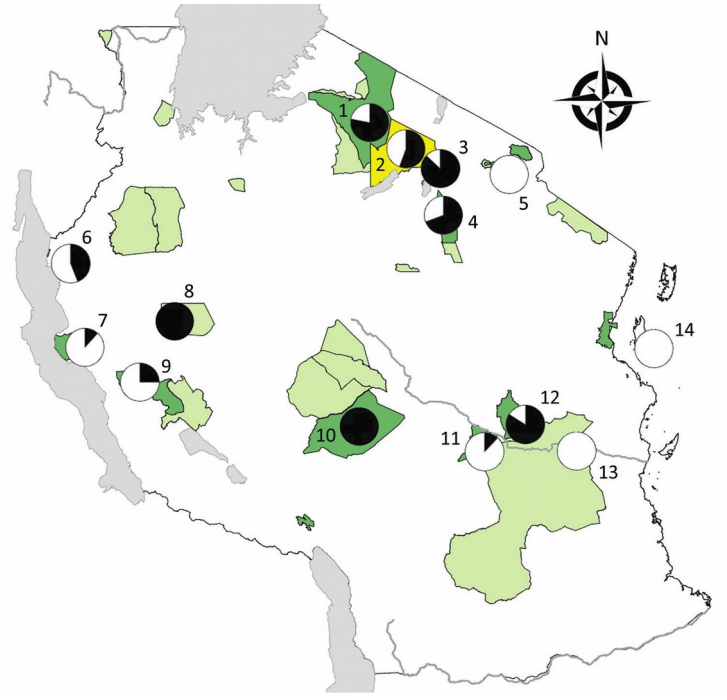

Figure 1. Protected areas and sites where free-ranging nonhuman primates (NHPs) were sampled in a study of *Treponema pallidum* infection, Tanzania. 1, Serengeti National Park (41 NHPs); 2, Ngorongoro Conservation Area (18 NHPs); 3, Lake Manyara National Park (38 NHPs); 4, Tarangire National Park (26 NHPs); 5, Arusha National Park (14 NHPs); 6, Gombe National Park (32 NHPs); 7, Mahale National Park (17 NHPs); 8, Issa Valley (2 NHPs); 9, Katavi National Park (12 NHPs); 10, Ruaha National Park (18 NHPs); 11, Udzungwa National Park (25 NHPs); 12, Mikumi National Park (25 NHPs); 13, Selous Game Reserve (8 NHPs); 14, Jozani-Chwaka Bay National Park–Masingini Forest on Unguja Island, Zanzibar (13 NHPs). Dark green indicates national parks; light green indicates game reserves; yellow indicates conservation area. Circle graphs: black, NHPs *T. pallidum*–positive (serology and/or PCR); white, NHPs T. pallidum–negative (serology and PCR). The map was produced with ArcMap version 10.0 (ESRI, Redlands, CA, USA) by using shape files available from ESRI (national boundary of Tanzania, water bodies of Africa, main rivers of Africa). The shape files of the conservation areas of Tanzania were provided by the Tanzania National Park Authority and are available free from http://www.arcgis.com/home/item.html?id = 9b06fe723ad14991b30b1b85953224c1. Prevalence circles were generated using Excel version 15.38 (Microsoft, Redmond, WA, USA).

Denmark) and appropriate projectiles. Immobilized NHPs were continuously observed for vital parameters such as respirations, pulse frequency, and internal body temperature. We monitored pulse frequency and blood oxygen saturation using a Nellcor OxiMax N65 Pulse Oximeter (Tyco Healthcare Deutschland GmbH, Neustadt, Germany). Anesthetized animals underwent a standardized health check with special focus on skin lesions. We collected whole blood from the femoral vein using an S-Monovette closed blood collection system (Sarstedt, Nümbrecht, Germany) mounted with a 20G needle. We collected two 9-mL serum tubes under aseptic conditions. We then centrifuged serum tubes at 55,000 relative centrifugation force for 15 min, transferred serum into cryovials, and stored the vials in liquid nitrogen. In animals with skin lesions, we took a 6-mm biopsy from the skin ulcer using a sterile dermal biopsy punch. From each animal (and ulcer), we preserved tissue samples in lysis buffer (10 mmol/L Tris [pH 8.0], 0.1 EDTA, and 0.5% sodium dodecyl sulfate).

We treated animal wounds with Silverspray (Silver Aluminum Aerosol; Henry Schein, Langen, Germany) and allowed animals to recover under close supervision. Samples were temporarily stored at –80°C at the Tanzania Wildlife Research Institute headquarters (Arusha, Tanzania). Aliquots were exported to the German Primate Center (Göttingen, Germany) for further analysis and additional confirmation.

Serologic Testing

We used a commercially available treponemal test (ESPLINE TP; Fujirebio Diagnostics, Hannover, Germany) to check all serum samples for *T. pallidum* antibodies. The assay has been validated for use in baboons (*12*), where it performed with 97.7% (95% CI 87.7%–99.9%) sensitivity and 96.0% (95% CI 79.7%–99.9%) specificity. We tested serum samples on the day of sampling and operated and interpreted test cassettes according to the manufacturer's guidance.

DNA Extraction from Skin Tissue

We extracted DNA following the standard protocol of the QIAmp DNA Mini Kit (QIAGEN, Hilden, Germany), with some minor modifications. In brief, we cut ≈25 mg tissue into small pieces and incubated in 180 µL lysis buffer, in which the sample had been stored since collection. After adding 20 µL proteinase K, samples were digested overnight at 56°C and 900 rpm (Thermomixer Comfort; Eppendorf, Hamburg, Germany). We added an additional washing step using 300 µL AW1 buffer and eluted the DNA twice with 100 µL AE buffer. We further purified extracted DNA using glycogen precipitation according to the protocol published in Knauf et al. (*13*).

TP_0105 (*polA*) Amplification and Sequencing

We performed PCR targeting the polymerase 1 gene (*TP_0105*, *polA*) of *T. pallidum* by using primers designed by Liu et al. (*14*). This assay has a reported sensitivity of 95.8% and a specificity of 95.7% and has been demonstrated to segregate pathogenic *T. pallidum* subspecies from nonpathogenic treponemes, other spirochetes, and 59 species of bacteria and viruses including those causing genital ulcers in humans (*14*). The 50-µL reaction volume comprised 25 µL 2 × Universe High-Fidelity Hot Start DNA Polymerase Master Mix (Biotool, Munich, Germany), 17 µL RNAase free water, 2 µL of each 10 µmol/L primer, 1 µL DNA polymerase (1 U/µL), 1 µL of 10 mmol/L each dNTP, and 2 µL template DNA, independent of DNA concentration. We conducted amplification in a SensoQuest Labcycler using the following thermocycling conditions: predenaturation at 95°C for 3 min, followed by 50 cycles each with 95°C for 15 s, 60°C for 15 s, and 72°C for 30 s. The profile was completed with a postextension step at 72°C for 5 min and indefinite cooling of the PCR product at 8°C. All *polA* PCR products were run on a 1% agarose gel to check for PCR performance and correct amplicon size. We gel extracted a representative subset of the PCR products (n = 19), purified with the QIAGEN Gel Extraction Kit (QIAGEN), and Sanger sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the amplification primers. Sequencing was performed by Seqlab Sequence Laboratories (Microsynth, Göttingen, Germany).

TP_0574 (*tp47*) Quantitative PCR

We performed TaqMan real-time PCR targeting a 132-bp fragment of the *TP_0574* gene. Primers and probe used were published elsewhere (*15*). The reaction encompassed 10 µL TaqMan Universal MasterMix II (no Uracil-N glycosylase; Applied Biosystems) and 1.8 µL of each 10 µmol/L primer and the probe. Total genomic DNA concentration added to each reaction was normalized to 100 ng. Molecular-grade water was used to adjust the reaction volume to 20 µL. Cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, followed by 50 cycles each at 95°C for 15 s and 60°C for 60 s. Reactions were run on a StepOnePlus Real-Time PCR System (Applied Biosystems). We measured all samples as triplicates and analyzed data using StepOne version 2.3 software (Applied Biosystems).

TP_0619 Amplification and Sequencing

We performed PCR targeting the *TP_0619* gene of *T. pallidum* to distinguish infection with TPE or *T. pallidum* subsp. *endemicum* (TEN) strains from infection with *T. pallidum* subsp. *pallidum* (TPA) strains. At this locus, TPA differs from TPE and TEN in ≥73 positions (online Technical

Appendix 1 Figure). We used primers 5′-TTACCCAGACATTTTTCTCCACATA-3′ and 5′-TACAAGCTCCCACAATGCCA-3′ to amplify a 608-bp fragment. The PCR conditions and working steps were identical to the PCR targeting the *polA* gene, except that the annealing temperature was adjusted to 55°C.

Data Analysis

We performed statistical analyses using GraphPad Prism version 7.0c (GraphPad Software, La Jolla, CA, USA), and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). We compared variables such as the presence of *T. pallidum* antibodies and clinical manifestations per species by using $2 \times 2 \times n$ contingency tables and a 2-tailed Fisher exact test. We used a χ^2 test to compare the outcome of >2 sampling sites using $n \times 2$ contingency tables. Proportions were tested at a critical probability of 0.05 and 95% CI. We considered $p \leq 0.05$ as statistically significant.

We analyzed and edited retrieved sequence data using 4Peaks 1.8 (http://www.nucleobytes.com) and SeaView 4.5.4 software (*16*). We compared sequences with respective orthologs available in GenBank using a standard nucleotide BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

NHP Species

We sampled 289 NHPs (Table) and confirmed previously reported *T. pallidum* infection in olive baboons at GNP (*5*,*17*,*18*), SNP (*3*), NCA (*3*), and LMNP (*3*,*6*,*7*). In addition, we report *T. pallidum* infection in yellow baboons, vervet monkeys, and blue monkeys in different regions of Tanzania (Table; Figure 1; online Technical Appendix 2, https://wwwnc.cdc.gov/EID/article/24/6/18-0037-Techapp2.xlsx).

The overall mean seropositivity of *T. pallidum* infection in the NHP samples was 53.3% (154/289). More female (82/135 [60.7%]) than male (72/154 [46.8%]) NHPs had *T. pallidum* antibodies. Overall, 35/45 (77.8%) vervet monkeys, 85/137 (62.0%) olive baboons, 33/75 (44.0%) yellow baboons and 1/15 (6.7%) blue monkeys had antibodies against the bacterium. Most (94 [61.0%]) of the 154 seropositive NHPs appeared healthy without any clinical skin lesions. The association between *T. pallidum* antibodies and skin ulceration was tested using 2-tailed Fisher exact test and was significant in olive baboons (n = 137; odds ratio [OR] 15.95 [95% CI 4.7–51.1]; p<0.0001) and yellow baboons (n = 75; OR 11.04 [95% CI 1.7–126.8]; p = 0.0185), but not in vervet (n = 45; OR ∞ [95% CI 0.0–1.0]; p = 0.0888) and blue monkeys (n = 15; OR 0.00 [95% CI 0.0–126.0]; p>0.9999 [dataset is provided in online Technical Appendix 2]). No *T. pallidum* antibodies were detected in the 10 Zanzibar red colobus, 3 Udzungwa red colobus, 2 Ugandan red colobus, and 2 red-tailed monkeys sampled. Moreover, none of these 4 species showed any kind of skin ulceration (Table).

Clinical Manifestations

Among the 156 *T. pallidum*–seropositive and/or PCR-positive NHPs (including 2 serologically negative but PCR-positive animals) and across the different sampling sites, we found anogenital ulcers associated with the infection (Figure 2, panel A) in 59.8% ± 23.9% of the yellow baboons (mean ± SEM, 6 investigated sites; data were analyzed as fraction of *T. pallidum*–infected animals with anogenital lesions per sampling site); 45.6% ± 16.2% of the olive baboons (mean ± SEM, 6 investigated sites); and 31.6% ± 9.4% of the infected vervet monkeys (mean ± SEM, 9 investigated sites). One of the 2 *T. pallidum*–infected blue monkeys showed anogenital skin ulceration; the second animal was clinically healthy. Orofacial lesions (Figure 2, panel B) were exclusively observed in

Table. Test results of *Treponema pallidum* infection in samples of free-ranging nonhuman primate species, Tanzania*

Species	No. (%)	Total/seropositive/skin lesion/PCR positive†					
		Positive			Negative		
		Total	Male	Female	Total	Male	Female
Olive baboon (*Papio anubis*)	137 (47.4)	86	34/34/12/12	52/51/31/30‡	51	29/0/1‡/NA	22/0/1‡/NA
Yellow baboon (*Papio cynocephalus*)	75 (26.0)	33	17/17/2/2	16/16/5/5	42	27/0/1§/NA	15/0/0/NA
Vervet monkey (*Chlorocebus pygerythrus*)	45 (15.6)	35	21/21/10/8‡	14/14/1/1	10	7/0/0/NA	3/0/0/NA
Blue monkey (*Cercopithecus mitis*)	15 (5.2)	2	1/0/1/1	1/1/0/0	13	8/0/0/NA	5/0/0/NA
Red-tailed monkey (*Cercopithecus ascanius*)	2 (0.7)	0	NA	NA	2	2/0/0/NA	NA
Zanzibar red colobus (*Piliocolobus kirkii*)	10 (3.5)	0	NA	NA	10	4/0/0/NA	6/0/0/NA
Udzungwa red colobus (*Piliocolobus gordonorum*)	3 (1.0)	0	NA	NA	3	2/0/0/NA	1/0/0/NA
Ugandan red colobus (*Piliocolobus tephrosceles*)	2 (0.7)	0	0/0/0/0	0/0/0/0	2	2/0/0/NA	NA
Total	289 (100.0)	156	73	83	133	81	52

*Results are based on the consensus of detected *T. pallidum* antibodies (ESPLINE TP) and PCR results of 3 independent gene targets (*polA*, *tp47*, and *TP_0619*). NA, not applicable.
†PCR was conducted only on animals with skin lesions.
‡No skin sample was available for some positive animals.
§Skin lesion at the genitalia most likely from fight; no tissue sample available.

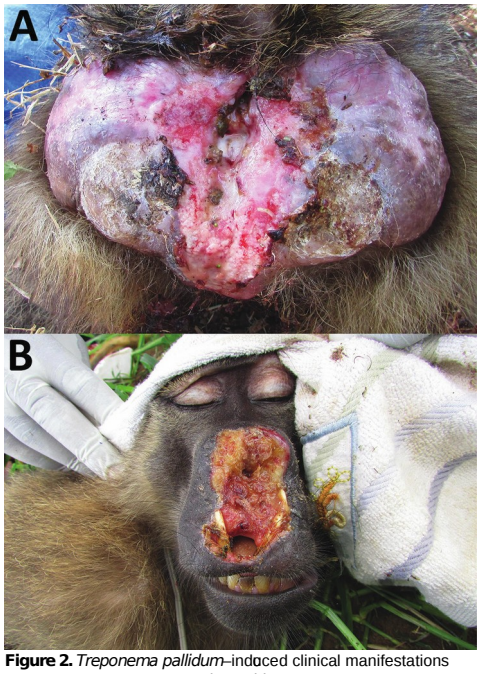

Figure 2. *Treponema pallidum*–induced clinical manifestations affecting olive baboons (*Papio anubis*), Tanzania. A). Lesions on the anogenital area of animal at Lake Manyara National Park. B) Facial lesions of animal at Tarangire National Park. Orofacial lesions were found only in olive baboons. A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/24/6/18-0037-F2.htm).

olive baboons at SNP, TNP, and LMNP, of which 2 olive baboons at TNP and 1 at SNP were included in our study. These animals represent 3.5% of the 86 *T. pallidum*–seropositive and/or PCR-positive sampled olive baboons. One animal from TNP had concurrent orofacial and anogenital skin ulcerations. We also observed these ulcerations in olive baboons at LMNP, although capture and sampling of these animals was not possible.

Geographic Distribution

Our results provide evidence for *T. pallidum* infection in NHPs at 11 of the 14 sites investigated (Figure 1; online Technical Appendix 2). The only sites where infection was not detected were ANP (14 NHPs), SGR (9 NHPs), and JCBNP (13 NHPs). We found *T. pallidum*–positive vervet monkeys in all areas where the species was examined (GNP, KNP, LMNP, MKNP, MNP, RNP, SNP, TNP, UNP) except for the 1 animal from Zanzibar (JCBNP). One PCR-positive and anogenital ulcerated blue monkey from LMNP had reproducibly negative serologic results. Because sampling was biased toward animals with skin lesions, we more objectively compared fied sites by analyzing data from animals that appeared to be clinically unaffected. Healthy-looking olive baboons were significantly more often *T. pallidum*–positive at LMNP (n = 6/6) than at any other sampling area in Tanzania where the species is present (ANP [n = 0/12], GNP [n = 8/23], NCA [1/9], SNP [n = 16/25], TNP [n = 12/17]; 6×2 contingency table: $\chi^2 = 30.15$, df = 5; $p<0.0001$). Likewise, clinically unaffected yellow baboons were significantly more often *T. pallidum*–infected at MKNP (n = 16/19) than at any of the other sampling areas in Tanzania where the species is present (KNP [n = 0/6], MNP [n = 0/10], RNP [n = 8/16], SGR [n = 0/7], UNP [n = 2/17]; 6×2 contingency table: $\chi^2 = 38.39$, df = 5; $p<0.0001$). In the vervet monkeys, we found no differences among sampling sites (GNP [n = 3/3], KNP [n = 2/5], LMNP [n = 1/2], MKNP [n = 2/3], MNP [n = 1/2], RNP [n = 4/4], SNP [n = 8/8], TNP [n = 3/6], JCBNP [n = 0/1]; 9×2 contingency table: $\chi^2 = 12.97$, df = 8; $p = 0.1130$), but sample size per site was low (online Technical Appendix 2).

Molecular Characterization of *T. pallidum* Samples

In the 65 animals with skin ulcers, we confirmed *T. pallidum* by amplification of a part of the *polA* gene (classic PCR) and/or the *tp47* locus (quantitative PCR; 59/60 animals tested positive; online Technical Appendix 2). For 5 animals, we did not perform PCR because of limited quantities of samples. All obtained sequences were identical. We deposited a representative sequence of the *polA* gene from a yellow baboon (16RUF8140716) in GenBank (accession no. MF627733). Of 58 tested animals, 56 were positive in the PCR targeting the *TP_0619* locus. For 7 NHPs, no PCR was performed because of sample limitations. Again, all 41 sequences obtained were identical. We deposited a representative sequence from a vervet monkey (4KNF2121016) in GenBank (accession no. MF754122). The haplotype was identical to those derived from TPE and TEN strains but different from TPA strains in ≥73 positions (online Technical Appendix 1 Figure).

Discussion

We confirmed *T. pallidum* infection in 4 free-ranging NHP species at 11 of 14 investigated sites in Tanzania. Our data for GNP must be interpreted with caution. GNP has a history of treating infected baboons with antimicrobial drugs (*17*), which might have affected prevalence rates and clinical manifestations. The finding that clinically unaffected olive baboons at LMNP, but also many animals at SNP and TNP, were infected with the bacterium (as indicated by serology; Table) shows that clinical manifestations are not representative of the actual prevalence of the disease. This finding is consistent with reports from an earlier

investigation of olive baboons at LMNP in 2007 (*6*) and in Guinea baboons (*Papio papio*) in the Niokolo Koba National Park, Senegal (*11*). In the context of human *T. pallidum* infection, where a latent stage is a key feature of infection (*19*) and which equally features positive serology in the absence of active skin lesions (*20*), this finding could argue for a similarity of disease progression in the NHP host. However, in the absence of long-term monitoring data for infected NHPs, relapsing cases, which would indicate the latent stage, cannot be identified, and standardized laboratory infection might be needed to obtain those data.

Although reduced susceptibility for *T. pallidum* infection is possible in some of the investigated species (colobines), it is likely that infection is not yet present because of behavioral and ecologic constraints between the infected and noninfected species. At least in a recent publication, a Ugandan red colobus was described with suspected active yaws-like lesions in Uganda (*21*). Consequently, we note that our sample size for colobines and red-tailed monkeys was insufficient. As a result, a conclusive evaluation on possible *T. pallidum* infection in these species was not possible. The same applied for sites where the number of infected NHPs was critically low, for example, UNP and MNP or the negative tested areas at ANP (14 animals), SGR (9 animals), and JCBNP (13 animals), as well as the NCA crater region where all 8 olive baboons were tested negative. We found *T. pallidum*–infected vervet monkeys with and without skin ulcers in 9 of the 10 sites where the species has been investigated. This finding and the larger number and geographic extent of *T. pallidum* infection in *Chlorocebus* sp. (*4*,*11*,*22*–*24*) deserve further attention in prospective studies, especially in areas where the species is present but has not yet been tested.

All *T. pallidum*–positive NHPs in this study revealed a *TP_0619* sequence that points toward infection with either TPE or TEN strains (online Technical Appendix 1 Figure). In the context of the geographic distribution of TEN strains (dry areas in Sahelian Africa and western Asia) (*25*) as well as the information obtained from the whole-genome sequences of the Tanzanian simian strains LMNP1 and 2 (S. Knauf et al., unpub. data, https://www.biorxiv.org/content/early/2017/05/10/135491), which are considered TPE strains, we assume that TPE is the dominant, if not exclusive, *T. pallidum* subspecies infecting Tanzanian NHPs. Further clarification will be achieved when multilocus strain typing data and whole-genome sequence data of the NHP samples become available.

In humans, TPE is mainly transmitted by direct skin-to-skin contact (*26*). A possible important alternate route of infection has been discussed through the involvement of flies as a vector (*27*,*28*). Although both options are at least theoretically possible for NHPs (*13*), direct contact should be considered the most likely way of intraspecies and interspecies transmission. Such transmission is further supported by reports of the close association and interaction (play, fight, or hunt) among different NHP species (*29*–*31*). Again, multilocus strain typing and whole-genome sequence data of the strains infecting NHPs in Tanzania are likely to contribute to a better understanding of host–pathogen coevolution and will provide details of the relatedness of the *T. pallidum* subspecies that infect the different NHP taxa.

Human yaws is known to be endemic to 13 countries, but Tanzania is among the 76 countries with a known history of the disease that lack recent epidemiologic data (*2*). More precisely, the disease was reported to be endemic in humans in the western areas along Lake Tanganyika and in southern Tanzania (*32*). Extensive elimination efforts decreased the reported incidence of human yaws in Tanzania from 120,000 cases in 1927 to 52,000 in 1950 (*33*) and 71 in 1978 (https://web.gideononline.com). At the same time, the wide distribution of *T. pallidum* infection in NHP on Tanzania's mainland (*7*) and the chronic infection with locally high prevalence rates (e.g., LMNP [*6*]) suggest the pathogen has been present in the respective NHP populations for at least several decades. However, current data are insufficient to develop a conclusive biogeographic scenario about the origin and spread of the infection. The first published report on *T. pallidum* infection in NHPs in 1989 (*5*) involved olive baboons at GNP. Although this is no evidence for the origin of *T. pallidum* infection in NHPs in Tanzania, it is interesting in the context of a possible anthropozoonotic introduction of the disease. GNP is in the region that has been historically classified as an area to which human yaws in Tanzania is endemic (*33*). Furthermore, GNP is close to the Democratic Republic of the Congo, a country that still reports cases of human yaws (*34*). However, all of this is speculative, and whole-genome data are needed from NHPs and human strains from the same area to provide a deeper understanding on the origin and transmission of *T. pallidum* in NHPs in Tanzania.

In a larger context, neighboring countries currently do not report NHPs with *T. pallidum*–confirmed skin lesions, although animals from East Africa (not further classified) (*22*) and Kenya (*3*) have tested serologically positive. Because *T. pallidum* infection in NHPs in Africa is widespread (*1*), further investigations should specifically include more East Africa countries, particularly those that share their borders with Tanzania.

We showed that *T. pallidum* infection in NHPs in Tanzania is geographically widespread and present in several Old World monkey species, namely olive and yellow baboons, vervet monkeys, and blue monkeys (hypothesis A). We identified the pathogen in almost all investigated sites

covering large parts of Tanzania's mainland (hypothesis B) and showed that NHPs in Tanzania are most likely infected by TPE strains. Nevertheless, our overall sample size does not permit a conclusive statement on *T. pallidum* prevalence in NHPs at any of the sampled sites. Further studies on the spatial distribution of NHP infection with *T. pallidum* and advanced genetic characterization of simian strains are crucial for identifying NHPs as a possible reservoir for human infection (*35*). In light of the data and for a sustainable eradication of human yaws, a One Health approach in which animal and human health is investigated (*36*) is needed.

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About the Author

Mr. Chuma is a veterinarian and PhD student at the Sokoine University of Agriculture in Tanzania. His research focuses on the epidemiology and characterization of nonhuman primate infection with *T. pallidum* under the aspect of One Health.

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Widespread *Treponema pallidum* Infection in Nonhuman Primates, Tanzania

Technical Appendix 1

Sample Size Calculation

We used FreeCalc, a calculator for sample size for freedom testing with imperfect test available through http://epitools.ausvet.com.au/content.php?page=FreeCalc2. The tool is based on the methods published by Cameron and Baldock in 1998 (*1*) and is used to calculate the required sample size and cut point for testing to demonstrate population freedom from disease using imperfect tests and allowing for small populations. Two assumptions were tested. First, we used the disease prevalence known from baboons at Lake Manyara National Park (*2*) and second, we tested for the scenario with a much lower disease prevalence (25%) (Technical Appendix 1 Table).

Interpretation

If a random sample of 4 units is taken from a population of 1,000 and $\leq$1 reactors are found, the probability that the population is diseased at a prevalence of 0.85 is 0.0145.

If a random sample of 21 units is taken from a population of 1,000 and $\leq$2 reactors are found, the probability that the population is diseased at a prevalence of 0.25 is 0.0444.

Ethics Statement

Free-ranging nonhuman primates (NHPs) were chemically immobilized and sampled in accordance with the requirements of the relevant guidelines and regulations, in particular the Tanzania Veterinary Act No. Sixteen of 2003 and Tanzania Wildlife Research Institute's (TAWIRI) Guidelines for Conducting Wildlife Research (2012; http://tawiri.or.tz/wp-content/uploads/2017/05/Wildlife-research-guideline.pdf). Respective permits for wildlife-

protected areas were issued by the Commission for Science and Technology in Tanzania (2015–89-NA-2014–228), Ministry for Natural Resources and Tourism (Wildlife Division, HA.403/563/01B/90, 178/606/01/115 and HA.178/606/01/6), Tanzania National Parks (TNP/HQ/C.10/13), and Ngorongoro Conservation Area Authority (NCAA/D/240/Vol.XXV/130) as well as the Revolutionary Government of Zanzibar through the second Vice-President's Office (Zanzibar Research Committee OMPR/M.95/C.6/2/Vol.IV/60). The study methods including the animal handling protocols were reviewed and approved by the Animal Welfare and Ethics Committee of the German Primate Center (E10–17) and the Vice Chancellor of Sokoine University of Agriculture (SUA/ADM/R.1/8). We applied "Good Veterinary Practice" rules to all procedures where animals were handled. Registered veterinarians immobilized NHPs and closely monitored anesthetized animals until they fully recovered.

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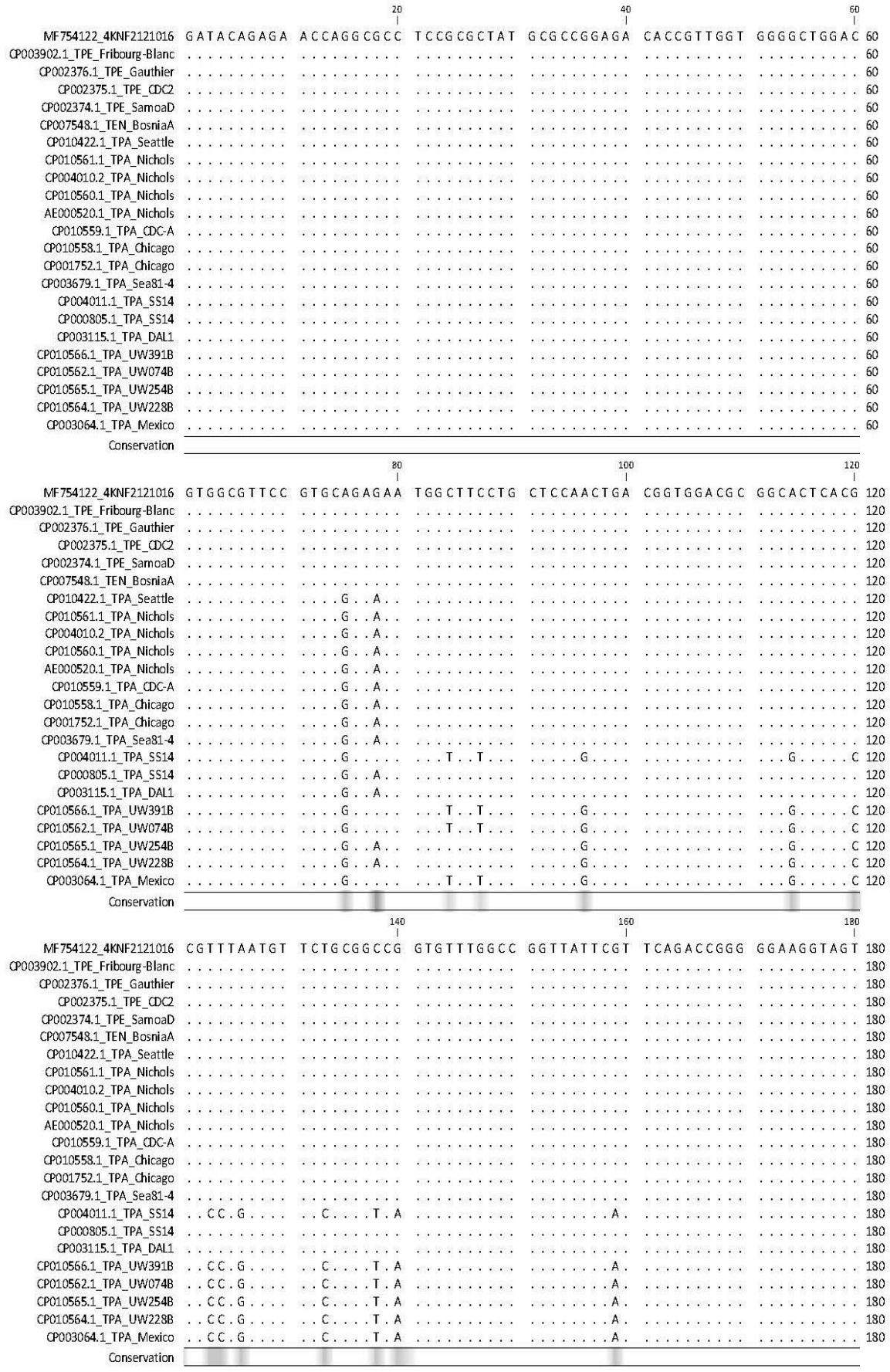
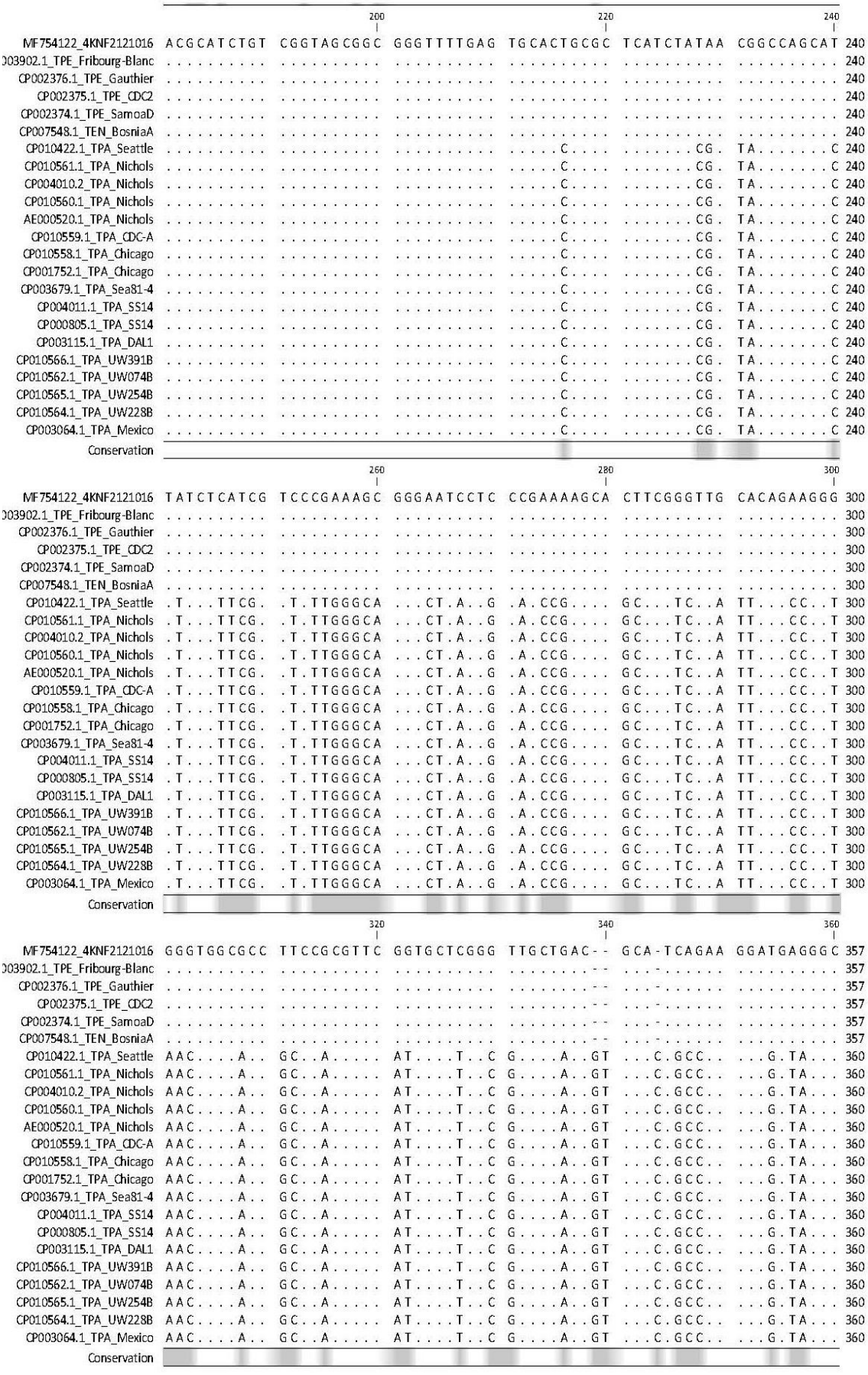
Technical Appendix 1 Table. Sample size calculations of free-ranging nonhuman primates included in the study of *Treponema pallidum* Infection, Tanzania.

Variable and results	*T. pallidum* prevalence 85%	*T. pallidum* prevalence 25%
Input variable		
Test sensitivity*	0.98	0.98
Test specificity*	0.96	0.96
Population size	1,000	1,000
Design prevalence	0.85	0.25
Diseased elements	850	250
Analysis method	Modified hypergeometric exact	Modified hypergeometric exact
Target Type I error	0.05	0.05
Target Type II error	0.05	0.05
Population threshold for infinite probability formula	10,000	10,000
Maximum sample size	100	100
Results		
Required sample size	4	21
Cut-point number of positives	1	2
Type I error	0.0145	0.0444
Type II error	0.0091	0.0497
Population-level sensitivity	0.9855	0.9556
Population-level specificity	0.9909	0.9503

*Espline TP (*3*).

Technical Appendix 1 Figure. Alignment of the *TP_0619* sequence data.

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CP002376.1_TPE_Gauthier 240
CP002375.1_TPE_CDC2 240
CP002374.1_TPE_SamoaD 240
CP007548.1_TEN_BosniaA 240
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CP000805.1_TPA_SS14 . . . . . C . . . . . CG. TA. . . . . C 240
CP003115.1_TPA_DAL1 . . . . . C . . . . . CG. TA. . . . . C 240
CP010566.1_TPA_UW391B . . . . . C . . . . . CG. TA. . . . . C 240
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CP010565.1_TPA_UW254B . . . . . C . . . . . CG. TA. . . . . C 240
CP010564.1_TPA_UW228B . . . . . C . . . . . CG. TA. . . . . C 240
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Conservation
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CP002376.1_TPE_Gauthier 300
CP002375.1_TPE_CDC2 300
CP002374.1_TPE_SamoaD 300
CP007548.1_TEN_BosniaA 300
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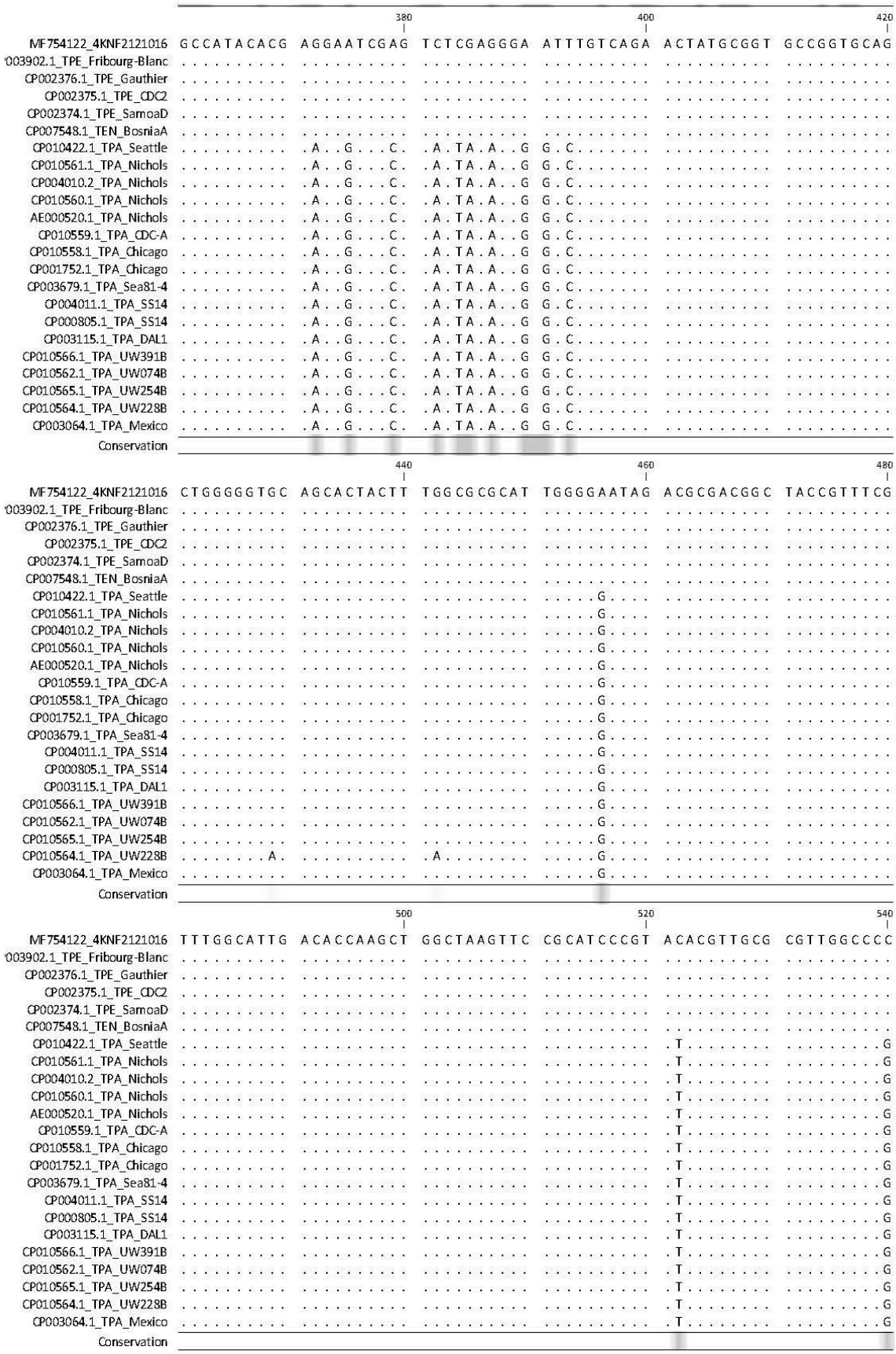
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AE000520.1_TPA_Nichols G 480
CP010559.1_TPA_CDC-A G 480
CP010558.1_TPA_Chicago G 480
CP001752.1_TPA_Chicago G 480
CP003679.1_TPA_Sea81-4 G 480
CP004011.1_TPA_SS14 G 480
CP000805.1_TPA_SS14 G 480
CP003115.1_TPA_DAL1 G 480
CP010566.1_TPA_UW391B G 480
CP010562.1_TPA_UW074B G 480
CP010565.1_TPA_UW254B G 480
CP010564.1_TPA_UW228B A. A. G 480
CP003064.1_TPA_Mexico G 480
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520
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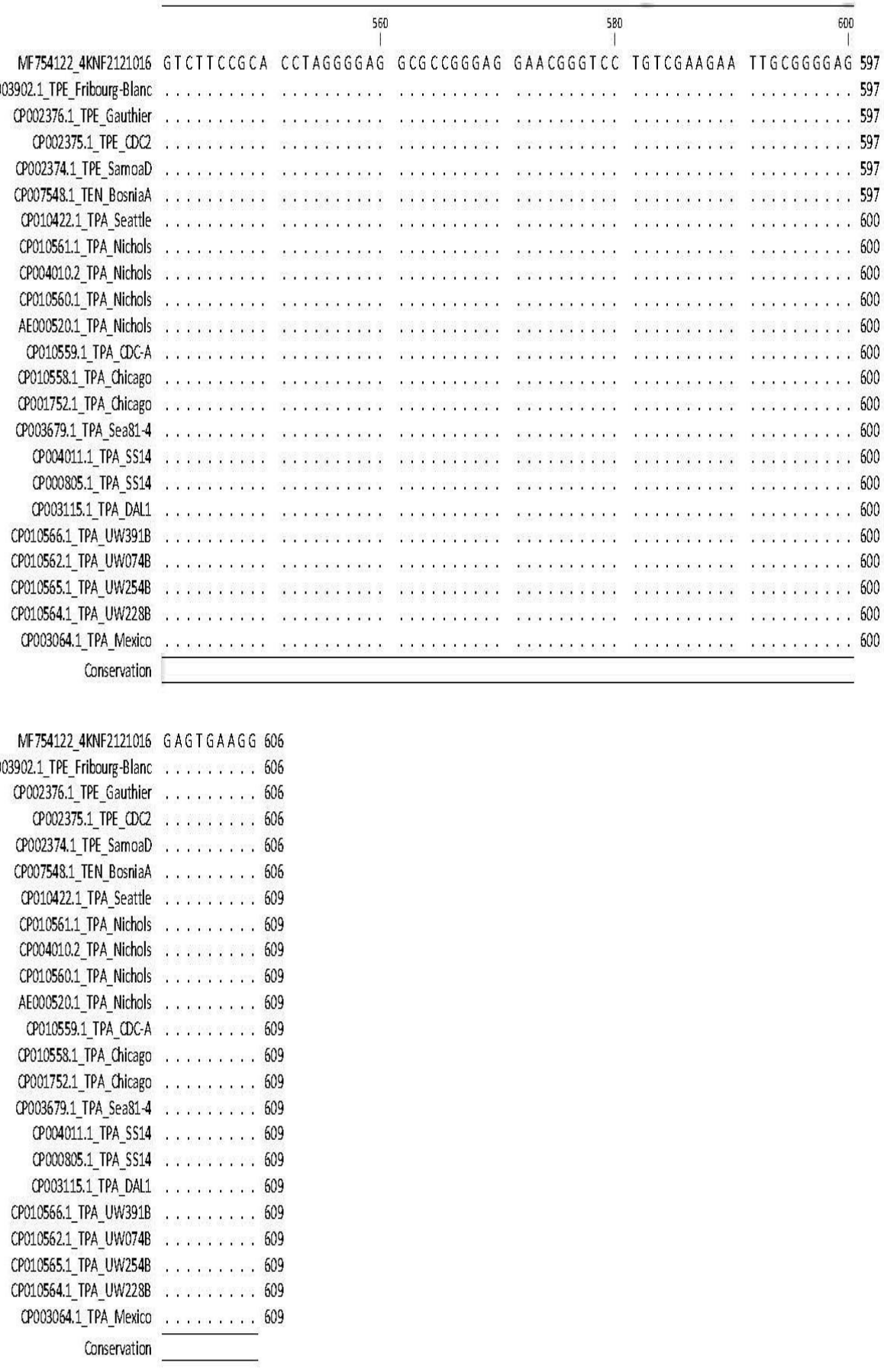
CP002376.1_TPE_Gauthier 537
CP002375.1_TPE_CDC2 537
CP002374.1_TPE_SamoaD 537
CP007548.1_TEN_BosniaA 537
CP010422.1_TPA_Seattle T G 540
CP010561.1_TPA_Nichols T G 540
CP004010.2_TPA_Nichols T G 540
CP010560.1_TPA_Nichols T G 540
AE000520.1_TPA_Nichols T G 540
CP010559.1_TPA_CDC-A T G 540
CP010558.1_TPA_Chicago T G 540
CP001752.1_TPA_Chicago T G 540
CP003679.1_TPA_Sea81-4 T G 540
CP004011.1_TPA_SS14 T G 540
CP000805.1_TPA_SS14 T G 540
CP003115.1_TPA_DAL1 T G 540
CP010566.1_TPA_UW391B T G 540
CP010562.1_TPA_UW074B T G 540
CP010565.1_TPA_UW254B T G 540
CP010564.1_TPA_UW228B T G 540
CP003064.1_TPA_Mexico T G 540
Conservation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CP002375.1_TPE_CDC2 597
CP002374.1_TPE_SamoaD 597
CP007548.1_TEN_BosniaA 597
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CP003064.1_TPA_Mexico 609
Conservation

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Technical Appendix 2. Dataset of all free-ranging nonhuman primates included into the study of *Treponema pallidum* infection, Tanzania. Sampling site abbreviations as indicated in the main text and Figure 1. AG, anogential; CNA, clinically nonaffected; NA, not applicable; OF, orofacial. Geographic coordinates in decimal degrees.

S/No.	IDNo.	Geolocation	Sex	Numerical ID code	Samplingdate	Species (common name)	Species (scientific name)	Latidude	Longitude	Espline TPtest	PCR (polA/tp47)	TP_negative6positive9	Lesion type
1	13	ANP	F	1	050116	Blue monkey	*Cercopithecus mitis*	-3.23703	36.85680	negative	NA	NA	CNA
2	14	ANP	F	1	050116	Blue monkey	*Cercopithecus mitis*	-3.23708	36.85688	negative	NA	NA	CNA
3	01	ANP	M	1	060815	Olive baboon	*Papio anubis*	-3.23691	36.85287	negative	NA	NA	CNA
4	02	ANP	M	1	080815	Olive baboon	*Papio anubis*	-3.23695	36.85294	negative	NA	NA	CNA
5	03	ANP	M	1	080815	Olive baboon	*Papio anubis*	-3.23696	36.85302	negative	NA	NA	CNA
6	04	ANP	M	1	030116	Olive baboon	*Papio anubis*	-3.22495	36.88508	negative	NA	NA	CNA
7	05	ANP	F	1	030116	Olive baboon	*Papio anubis*	-3.23438	36.85582	negative	NA	NA	CNA
8	06	ANP	M	1	030116	Olive baboon	*Papio anubis*	-3.23418	36.85602	negative	NA	NA	CNA
9	07	ANP	M	1	040116	Olive baboon	*Papio anubis*	-3.22528	36.88463	negative	NA	NA	CNA
10	08	ANP	M	1	040116	Olive baboon	*Papio anubis*	-3.22523	36.88447	negative	NA	NA	CNA
11	09	ANP	F	1	040116	Olive baboon	*Papio anubis*	-3.27550	36.89670	negative	NA	NA	CNA
12	10	ANP	M	1	040116	Olive baboon	*Papio anubis*	-3.23355	36.85050	negative	NA	NA	CNA
13	11	ANP	F	1	040116	Olive baboon	*Papio anubis*	-3.23380	36.85082	negative	NA	NA	CNA
14	12	ANP	M	1	050116	Olive baboon	*Papio anubis*	-3.23622	36.85692	negative	NA	NA	CNA
15	37	GNP	M	1	260916	Blue monkey	*Cercopithecus mitis*	-4.67201	29.62492	negative	NA	NA	CNA
16	34	GNP	F	1	240916	Vervet monkey	*Chlorocebus pygerythrus*	-4.62538	29.63649	positive	NA	NA	CNA
17	35	GNP	F	1	240916	Vervet monkey	*Chlorocebus pygerythrus*	-4.62611	29.63636	positive	NA	NA	CNA
18	36	GNP	M	1	250916	Vervet monkey	*Chlorocebus pygerythrus*	-4.62792	29.63461	positive	NA	NA	CNA
19	01	GNP	M	1	200916	Olive baboon	*Papio anubis*	-4.63912	29.63054	positive	NA	NA	CNA
20	02	GNP	M	1	200916	Olive baboon	*Papio anubis*	-4.63918	29.63076	positive	NA	NA	CNA
21	03	GNP	F	8	200916	Olive baboon	*Papio anubis*	-4.63888	29.6307	positive	positive	positive	AG
22	04	GNP	F	1	200916	Olive baboon	*Papio anubis*	-4.63915	29.63101	positive	NA	NA	CNA
23	07	GNP	M	1	210916	Olive baboon	*Papio anubis*	-4.66637	29.62385	negative	NA	NA	CNA
24	08	GNP	M	1	210916	Olive baboon	*Papio anubis*	-4.66618	29.62386	negative	NA	NA	CNA
25	09	GNP	F	1	210916	Olive baboon	*Papio anubis*	-4.66689	29.62393	negative	NA	NA	CNA
26	10	GNP	F	1	210916	Olive baboon	*Papio anubis*	-4.66666	29.62395	negative	NA	NA	CNA
27	11	GNP	M	1	210916	Olive baboon	*Papio anubis*	-4.73334	29.60914	negative	NA	NA	CNA
28	12	GNP	M	1	210916	Olive baboon	*Papio anubis*	-4.73334	29.6091	negative	NA	NA	CNA
29	13	GNP	M	1	210916	Olive baboon	*Papio anubis*	-4.73325	29.60915	negative	NA	NA	CNA
30	14	GNP	F	1	210916	Olive baboon	*Papio anubis*	-4.73343	29.60956	negative	NA	NA	CNA
31	19	GNP	M	2	220916	Olive baboon	*Papio anubis*	-4.67816	29.62085	positive	positive	positive	AG
32	20	GNP	F	5	220916	Olive baboon	*Papio anubis*	-4.67811	29.62168	positive	positive	positive	AG
33	21	GNP	F	1	220916	Olive baboon	*Papio anubis*	-4.67817	29.62083	positive	NA	NA	CNA
34	22	GNP	M	1	220916	Olive baboon	*Papio anubis*	-4.67841	29.62178	negative	NA	NA	CNA
35	23	GNP	F	1	220916	Olive baboon	*Papio anubis*	-4.62763	29.63499	positive	NA	NA	CNA
36	24	GNP	F	1	220916	Olive baboon	*Papio anubis*	-4.62806	29.6344	positive	NA	NA	CNA
37	25	GNP	M	1	220916	Olive baboon	*Papio anubis*	-4.62779	29.63467	positive	NA	NA	CNA
38	26	GNP	M	1	230916	Olive baboon	*Papio anubis*	-4.67113	29.62853	negative	NA	NA	CNA
39	27	GNP	F	1	230916	Olive baboon	*Papio anubis*	-4.67127	29.6281	negative	NA	NA	CNA
40	28	GNP	F	1	230916	Olive baboon	*Papio anubis*	-4.67092	29.62859	negative	NA	NA	CNA
41	29	GNP	M	1	230916	Olive baboon	*Papio anubis*	-4.67121	29.6279	negative	NA	NA	CNA
42	30	GNP	F	2	230916	Olive baboon	*Papio anubis*	-4.67112	29.62763	negative	NA	NA	AG
43	31	GNP	M	2	230916	Olive baboon	*Papio anubis*	-4.67244	29.63111	negative	NA	NA	AG
44	32	GNP	F	1	230916	Olive baboon	*Papio anubis*	-4.67257	29.63104	negative	NA	NA	CNA
45	33	GNP	M	1	230916	Olive baboon	*Papio anubis*	-4.67255	29.63106	negative	NA	NA	CNA
46	38	GNP	M	1	260916	Olive baboon	*Papio anubis*	-4.62871	29.63471	positive	NA	NA	CNA
47	01	Issa	F	5	101016	Yellow baboon	*Papio cynocephalus*	-5.50952	30.56185	positive	positive	positive	AG
48	02	Issa	F	8	101016	Yellow baboon	*Papio cynocephalus*	-5.5112	30.56357	positive	positive	positive	AG
49	02	JCBNP	M	1	100517	Blue monkey	*Cercopithecus mitis*	-6.27491	39.41982	negative	NA	NA	CNA
50	08	JCBNP	F	1	120517	Blue monkey	*Cercopithecus mitis*	-6.27336	39.41919	negative	NA	NA	CNA
51	13	JCBNP	M	1	130517	Vervet monkey	*Chlorocebus pygerythrus*	-6.10050	39.24395	negative	NA	NA	CNA
52	01	JCBNP	F	1	100517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.27522	39.42050	negative	NA	NA	CNA
53	03	JCBNP	F	1	100517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.2749	39.41984	negative	NA	NA	CNA
54	04	JCBNP	M	1	110517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.27286	39.41867	negative	NA	NA	CNA
55	05	JCBNP	F	1	110517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.27103	39.41919	negative	NA	NA	CNA
56	06	JCBNP	F	1	110517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.27305	39.41847	negative	NA	NA	CNA
57	07	JCBNP	M	1	120517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.27301	39.41919	negative	NA	NA	CNA
58	09	JCBNP	M	1	120517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.27042	39.41968	negative	NA	NA	CNA
59	10	JCBNP	M	1	120517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.27107	39.41913	negative	NA	NA	CNA
60	11	JCBNP	F	1	120517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.27098	39.41926	negative	NA	NA	CNA
61	12	JCBNP	F	1	120517	Zanzibar red colobus	*Piliocolobus kirkii*	-6.27189	39.42117	negative	NA	NA	CNA
62	01	KNP	M	1	111016	Vervet monkey	*Chlorocebus pygerythrus*	-6.633	31.14212	negative	NA	NA	CNA

S/No.	ID No.	Geo location	Sex	Numerical ID code	Sampling date	Species (common name)	Species (scientific name)	Latidude	Longitude	Espline TP test	PCR (polA/tp47)	TP_negative6p ositive9	Lesion type
63	02	KNP	M	1	111016	Vervet monkey	*Chlorocebus pygerythrus*	-6.63299	31.14156	negative	NA	NA	CNA
64	03	KNP	F	1	121016	Vervet monkey	*Chlorocebus pygerythrus*	-6.64316	31.13849	positive	NA	NA	CNA
65	04	KNP	F	2	121016	Vervet monkey	*Chlorocebus pygerythrus*	-6.64327	31.13878	positive	positive	positive	AG
66	11	KNP	M	1	131016	Vervet monkey	*Chlorocebus pygerythrus*	-6.68696	31.04111	negative	NA	NA	CNA
67	12	KNP	M	1	131016	Vervet monkey	*Chlorocebus pygerythrus*	-6.6401	31.13964	positive	NA	NA	CNA
68	05	KNP	M	1	121016	Yellow baboon	*Papio cynocephalus*	-6.63357	31.14192	negative	NA	NA	CNA
69	06	KNP	M	1	121016	Yellow baboon	*Papio cynocephalus*	-6.63392	31.14341	negative	NA	NA	CNA
70	07	KNP	M	1	121016	Yellow baboon	*Papio cynocephalus*	-6.63679	31.14333	negative	NA	NA	CNA
71	08	KNP	M	1	121016	Yellow baboon	*Papio cynocephalus*	-6.63684	31.14306	negative	NA	NA	CNA
72	09	KNP	F	1	131016	Yellow baboon	*Papio cynocephalus*	-6.63295	31.14415	negative	NA	NA	CNA
73	10	KNP	M	1	131016	Yellow baboon	*Papio cynocephalus*	-6.63435	31.14276	negative	NA	NA	CNA
74	09	LMNP	M	2	180815	Blue monkey	*Cercopithecus mitis*	-3.37267	35.83918	negative	positive	positive	AG
75	31	LMNP	F	1	190317	Blue monkey	*Cercopithecus mitis*	-3.40118	35.82246	negative	NA	NA	CNA
76	35	LMNP	M	1	200317	Blue monkey	*Cercopithecus mitis*	-3.41001	35.82075	negative	NA	NA	CNA
77	38	LMNP	M	1	200317	Blue monkey	*Cercopithecus mitis*	-3.41051	35.82007	negative	NA	NA	CNA
78	39	LMNP	F	1	200317	Blue monkey	*Cercopithecus mitis*	-3.41052	35.81993	positive	NA	NA	CNA
79	40	LMNP	M	1	200317	Blue monkey	*Cercopithecus mitis*	-3.41080	35.82013	negative	NA	NA	CNA
80	32	LMNP	M	2	190317	Vervet monkey	*Chlorocebus pygerythrus*	-3.40139	35.82240	positive	positive	positive	AG
81	33	LMNP	M	2	190317	Vervet monkey	*Chlorocebus pygerythrus*	-3.41275	35.83824	positive	positive	negative	AG
82	34	LMNP	M	2	190317	Vervet monkey	*Chlorocebus pygerythrus*	-3.41202	35.83840	positive	positive	positive	AG
83	36	LMNP	F	1	200317	Vervet monkey	*Chlorocebus pygerythrus*	-3.41003	35.82076	positive	NA	NA	CNA
84	37	LMNP	M	1	200317	Vervet monkey	*Chlorocebus pygerythrus*	-3.41015	35.82090	negative	NA	NA	CNA
85	02	LMNP	M	5	290415	Olive baboon	*Papio anubis*	-3.40093	35.8281	positive	positive	negative	AG
86	03	LMNP	F	5	300415	Olive baboon	*Papio anubis*	-3.40789	35.82821	positive	positive	positive	AG
87	04	LMNP	F	2	020515	Olive baboon	*Papio anubis*	-3.47691	35.77997	negative	positive	positive	AG
88	04	LMNP	F	8	160815	Olive baboon	*Papio anubis*	-3.3901	35.82521	positive	positive	positive	AG
89	06	LMNP	F	5	170815	Olive baboon	*Papio anubis*	-3.44967	35.79568	positive	positive	positive	AG
90	07	LMNP	F	5	170815	Olive baboon	*Papio anubis*	-3.37627	35.83327	positive	positive	positive	AG
91	10	LMNP	F	8	190815	Olive baboon	*Papio anubis*	-3.2129	35.82632	positive	positive	positive	AG
92	11	LMNP	F	5	200815	Olive baboon	*Papio anubis*	-3.38924	35.82561	positive	positive	positive	AG
93	12	LMNP	F	2	210815	Olive baboon	*Papio anubis*	-3.38903	35.82445	positive	positive	positive	AG
94	13	LMNP	M	8	210815	Olive baboon	*Papio anubis*	-3.38853	35.82323	positive	positive	positive	AG
95	14	LMNP	F	5	220815	Olive baboon	*Papio anubis*	-3.40577	35.81279	positive	positive	positive	AG
96	15	LMNP	F	5	250815	Olive baboon	*Papio anubis*	-3.42487	35.81424	positive	positive	positive	AG
97	16	LMNP	M	5	250815	Olive baboon	*Papio anubis*	-3.41109	35.82048	positive	positive	positive	AG
98	17	LMNP	F	5	270815	Olive baboon	*Papio anubis*	-3.40309	35.82507	positive	positive	positive	AG
99	18	LMNP	F	5	270815	Olive baboon	*Papio anubis*	-3.4271	35.81178	positive	positive	positive	AG
100	19	LMNP	F	8	280815	Olive baboon	*Papio anubis*	-3.40273	35.81382	positive	positive	positive	AG
101	20	LMNP	F	5	280815	Olive baboon	*Papio anubis*	-3.37805	35.83225	positive	positive	positive	AG
102	21	LMNP	F	2	290815	Olive baboon	*Papio anubis*	-3.40755	35.83487	positive	positive	positive	AG
103	22	LMNP	F	5	290815	Olive baboon	*Papio anubis*	-3.39319	35.82323	positive	positive	positive	AG
104	23	LMNP	M	1	081215	Olive baboon	*Papio anubis*	-3.47642	35.7795	positive	NA	NA	CNA
105	24	LMNP	M	2	081215	Olive baboon	*Papio anubis*	-3.47645	35.77947	positive	positive	positive	AG
106	25	LMNP	F	1	081215	Olive baboon	*Papio anubis*	-3.47643	35.77948	positive	NA	NA	CNA
107	26	LMNP	M	1	081215	Olive baboon	*Papio anubis*	-3.47643	35.77949	positive	NA	NA	CNA
108	27	LMNP	F	1	081215	Olive baboon	*Papio anubis*	-3.47644	35.77946	positive	NA	NA	CNA
109	28	LMNP	M	1	081215	Olive baboon	*Papio anubis*	-3.47644	35.77946	positive	NA	NA	CNA
110	29	LMNP	M	1	180416	Olive baboon	*Papio anubis*	-3.69545	36.37630	positive	NA	NA	CNA
111	30	LMNP	F	5	190416	Olive baboon	*Papio anubis*	-3.62500	36.39062	positive	positive	positive	AG
112	24	MKNP	M	1	170715	Vervet monkey	*Chlorocebus pygerythrus*	-7.3404	37.11786	positive	NA	NA	CNA
113	25	MKNP	M	5	170715	Vervet monkey	*Chlorocebus pygerythrus*	-7.34041	37.11789	positive	negative	NA	AG
114	28	MKNP	F	1	190715	Vervet monkey	*Chlorocebus pygerythrus*	-7.34033	37.11792	positive	NA	NA	CNA
115	30	MKNP	M	2	190715	Vervet monkey	*Chlorocebus pygerythrus*	-7.3404	37.11799	positive	NA	NA	AG
116	31	MKNP	F	1	190715	Vervet monkey	*Chlorocebus pygerythrus*	-7.34053	37.11789	negative	NA	NA	CNA
117	05	MKNP	M	1	130715	Yellow baboon	*Papio cynocephalus*	-7.36738	37.04478	positive	NA	NA	CNA
118	06	MKNP	M	1	130715	Yellow baboon	*Papio cynocephalus*	-7.34048	37.11778	positive	NA	NA	CNA
119	07	MKNP	M	1	140715	Yellow baboon	*Papio cynocephalus*	-7.35945	37.05548	positive	NA	NA	CNA
120	08	MKNP	F	1	150715	Yellow baboon	*Papio cynocephalus*	-7.3371	37.11446	positive	NA	NA	CNA
121	09	MKNP	F	1	150715	Yellow baboon	*Papio cynocephalus*	-7.33353	37.11079	negative	NA	NA	CNA
122	10	MKNP	F	1	150715	Yellow baboon	*Papio cynocephalus*	-7.3113	37.17857	negative	NA	NA	CNA
123	11	MKNP	F	1	150715	Yellow baboon	*Papio cynocephalus*	-7.31127	37.17862	positive	NA	NA	CNA
124	12	MKNP	M	1	150715	Yellow baboon	*Papio cynocephalus*	-7.30988	37.17853	positive	NA	NA	CNA
125	13	MKNP	M	1	150715	Yellow baboon	*Papio cynocephalus*	-7.30651	37.17908	positive	NA	NA	CNA
126	14	MKNP	M	1	160715	Yellow baboon	*Papio cynocephalus*	-7.37108	37.03915	positive	NA	NA	CNA
127	15	MKNP	F	1	160715	Yellow baboon	*Papio cynocephalus*	-7.35723	37.09789	positive	NA	NA	CNA
128	16	MKNP	F	1	160715	Yellow baboon	*Papio cynocephalus*	-7.35721	37.09791	positive	NA	NA	CNA
129	17	MKNP	F	1	160715	Yellow baboon	*Papio cynocephalus*	-7.27378	37.18573	positive	NA	NA	CNA
130	18	MKNP	F	1	160715	Yellow baboon	*Papio cynocephalus*	-7.27382	37.18571	negative	NA	NA	CNA

S/No.	ID No.	Geo location	Sex	Numerical ID code	Samplingdate	Species (common name)	Species (scientific name)	Latidude	Longitude	Espline TP test	PCR (polA/tp47)	TP_negative6positive9	Lesion type
131	19	MKNP	M	1	170715	Yellow baboon	*Papio cynocephalus*	-7.34337	37.16545	positive	NA	NA	CNA
132	21	MKNP	F	1	170715	Yellow baboon	*Papio cynocephalus*	-7.33403	37.17136	positive	NA	NA	CNA
133	22	MKNP	F	1	170715	Yellow baboon	*Papio cynocephalus*	-7.33406	37.17131	positive	NA	NA	CNA
134	23	MKNP	M	1	170715	Yellow baboon	*Papio cynocephalus*	-7.33413	37.17145	positive	NA	NA	CNA
135	26	MKNP	F	1	180715	Yellow baboon	*Papio cynocephalus*	-7.33829	37.11698	positive	NA	NA	CNA
136	27	MKNP	M	5	180715	Yellow baboon	*Papio cynocephalus*	-7.37263	37.03685	positive	positive	positive	AG
137	03	MNP	M	1	300916	Red-tailed monkey	*Cercopithecus ascanius*	-6.11802	29.73056	negative	NA	NA	CNA
138	11	MNP	M	1	021016	Red-tailed monkey	*Cercopithecus ascanius*	-6.11671	29.73051	negative	NA	NA	CNA
139	06	MNP	M	1	300916	Vervet monkey	*Chlorocebus pygerythrus*	-6.11897	29.73023	negative	NA	NA	CNA
140	07	MNP	M	2	300916	Vervet monkey	*Chlorocebus pygerythrus*	-6.1187	29.73005	positive	positive	positive	AG
141	17	MNP	F	1	041016	Vervet monkey	*Chlorocebus pygerythrus*	-6.03082	29.74384	positive	NA	NA	CNA
142	01	MNP	M	1	300916	Yellow baboon	*Papio cynocephalus*	-6.11885	29.73054	negative	NA	NA	CNA
143	02	MNP	M	1	300916	Yellow baboon	*Papio cynocephalus*	-6.11899	29.72996	negative	NA	NA	CNA
144	04	MNP	M	1	300916	Yellow baboon	*Papio cynocephalus*	-6.11903	29.73032	negative	NA	NA	CNA
145	05	MNP	M	1	300916	Yellow baboon	*Papio cynocephalus*	-6.1192	29.73023	negative	NA	NA	CNA
146	08	MNP	M	1	011016	Yellow baboon	*Papio cynocephalus*	-6.12868	29.73221	negative	NA	NA	CNA
147	09	MNP	M	1	011016	Yellow baboon	*Papio cynocephalus*	-6.11735	29.73055	negative	NA	NA	CNA
148	10	MNP	M	1	021016	Yellow baboon	*Papio cynocephalus*	-6.11924	29.73086	negative	NA	NA	CNA
149	13	MNP	F	1	031016	Yellow baboon	*Papio cynocephalus*	-6.03667	29.74191	negative	NA	NA	CNA
150	14	MNP	F	1	041016	Yellow baboon	*Papio cynocephalus*	-6.03722	29.74063	negative	NA	NA	CNA
151	15	MNP	F	1	041016	Yellow baboon	*Papio cynocephalus*	-6.0371	29.74065	negative	NA	NA	CNA
152	12	MNP	M	1	031016	Uganda red colobus	*Piliocolobus tephrosceles*	-6.03037	29.74412	negative	NA	NA	CNA
153	16	MNP	M	1	041016	Uganda red colobus	*Piliocolobus tephrosceles*	-6.03116	29.74448	negative	NA	NA	CNA
154	01	NCA	M	1	130516	Olive baboon	*Papio anubis*	-3.22163	35.50538	negative	NA	NA	CNA
155	02	NCA	M	1	140516	Olive baboon	*Papio anubis*	-3.22716	35.50768	negative	NA	NA	CNA
156	03	NCA	M	1	140516	Olive baboon	*Papio anubis*	-3.22609	35.50586	negative	NA	NA	CNA
157	04	NCA	F	1	140516	Olive baboon	*Papio anubis*	-3.22653	35.50597	negative	NA	NA	CNA
158	05	NCA	F	1	150516	Olive baboon	*Papio anubis*	-3.15231	35.60008	negative	NA	NA	CNA
159	06	NCA	F	1	150516	Olive baboon	*Papio anubis*	-3.15229	35.6001	negative	NA	NA	CNA
160	07	NCA	M	1	160516	Olive baboon	*Papio anubis*	-3.19659	35.50362	negative	NA	NA	CNA
161	08	NCA	F	1	160516	Olive baboon	*Papio anubis*	-3.20085	35.50811	negative	NA	NA	CNA
162	09	NCA	F	8	170516	Olive baboon	*Papio anubis*	-3.29935	35.59225	positive	positive	positive	AG
163	10	NCA	M	8	170516	Olive baboon	*Papio anubis*	-3.29935	35.59226	positive	positive	positive	AG
164	11	NCA	F	1	210317	Olive baboon	*Papio anubis*	-3.30042	35.59241	positive	NA	NA	CNA
165	12	NCA	F	5	210317	Olive baboon	*Papio anubis*	-3.30111	35.59233	positive	positive	positive	AG
166	13	NCA	M	2	210317	Olive baboon	*Papio anubis*	-3.30071	35.59212	positive	positive	positive	AG
167	14	NCA	M	5	210317	Olive baboon	*Papio anubis*	-3.30116	35.59241	positive	positive	positive	AG
168	15	NCA	F	2	220317	Olive baboon	*Papio anubis*	-3.30278	35.59204	positive	positive	positive	AG
169	16	NCA	F	2	220317	Olive baboon	*Papio anubis*	-3.30221	35.59189	positive	positive	positive	AG
170	17	NCA	F	2	220317	Olive baboon	*Papio anubis*	-3.29973	35.59233	positive	positive	positive	AG
171	18	NCA	F	8	220317	Olive baboon	*Papio anubis*	-3.29973	35.59223	positive	positive	positive	AG
172	03	RNP	F	1	080716	Vervet monkey	*Chlorocebus pygerythrus*	-7.67948	34.93293	positive	NA	NA	CNA
173	04	RNP	F	1	080716	Vervet monkey	*Chlorocebus pygerythrus*	-7.67940	34.93318	positive	NA	NA	CNA
174	05	RNP	M	2	080716	Vervet monkey	*Chlorocebus pygerythrus*	-7.67930	34.93323	positive	positive	positive	AG
175	06	RNP	M	2	090716	Vervet monkey	*Chlorocebus pygerythrus*	-7.68097	34.93183	positive	positive	NA	AG
176	10	RNP	M	1	100716	Vervet monkey	*Chlorocebus pygerythrus*	-7.68147	34.92992	positive	NA	NA	CNA
177	11	RNP	F	1	100716	Vervet monkey	*Chlorocebus pygerythrus*	-7.68158	34.93013	positive	NA	NA	CNA
178	01	RNP	M	1	080716	Yellow baboon	*Papio cynocephalus*	-7.68330	34.92918	positive	NA	NA	CNA
179	02	RNP	F	1	080716	Yellow baboon	*Papio cynocephalus*	-7.68305	34.92935	positive	NA	NA	CNA
180	07	RNP	F	1	090716	Yellow baboon	*Papio cynocephalus*	-7.68278	34.93070	positive	NA	NA	CNA
181	08	RNP	M	1	100716	Yellow baboon	*Papio cynocephalus*	-7.67835	34.92912	positive	NA	NA	CNA
182	09	RNP	F	1	100716	Yellow baboon	*Papio cynocephalus*	-7.68085	34.92972	positive	NA	NA	CNA
183	12	RNP	M	1	100716	Yellow baboon	*Papio cynocephalus*	-7.68223	34.93252	positive	NA	NA	CNA
184	13	RNP	M	1	130716	Yellow baboon	*Papio cynocephalus*	-7.60178	34.902	positive	NA	NA	CNA
185	14	RNP	F	5	130716	Yellow baboon	*Papio cynocephalus*	-7.59978	34.90537	positive	positive	positive	AG
186	15	RNP	M	1	140716	Yellow baboon	*Papio cynocephalus*	-7.59988	34.90358	positive	NA	NA	CNA
187	16	RNP	F	8	140716	Yellow baboon	*Papio cynocephalus*	-7.5998	34.90375	positive	positive	positive	AG
188	17	RNP	M	5	140716	Yellow baboon	*Papio cynocephalus*	-7.59862	34.8844	positive	positive	positive	AG
189	18	RNP	F	2	140716	Yellow baboon	*Papio cynocephalus*	-7.59952	34.88442	positive	positive	positive	AG
190	01	SGR	M	1	270217	Yellow baboon	*Papio cynocephalus*	-8.46428	38.55282	negative	NA	NA	CNA
191	02	SGR	M	1	280217	Yellow baboon	*Papio cynocephalus*	-8.47159	38.54414	negative	NA	NA	CNA
192	03	SGR	M	1	10317	Yellow baboon	*Papio cynocephalus*	-8.44884	38.59344	negative	NA	NA	CNA
193	04	SGR	M	2	300317	Yellow baboon	*Papio cynocephalus*	-7.53590	37.76624	negative	NA	NA	AG
194	05	SGR	F	1	30317	Yellow baboon	*Papio cynocephalus*	-7.53548	37.76555	negative	NA	NA	CNA
195	06	SGR	M	1	50317	Yellow baboon	*Papio cynocephalus*	-7.74917	38.20233	negative	NA	NA	CNA
196	07	SGR	F	1	50317	Yellow baboon	*Papio cynocephalus*	-7.74555	38.19652	negative	NA	NA	CNA
197	08	SGR	M	1	50317	Yellow baboon	*Papio cynocephalus*	-7.74681	38.19705	negative	NA	NA	CNA
198	14	SNP	M	1	111115	Vervet monkey	*Chlorocebus pygerythrus*	-2.46187	34.80593	positive	NA	NA	CNA

S/No.	ID No.	Geolocation	Sex	Numerical ID code	Sampling date	Species (common name)	Species (scientific name)	Latidude	Longitude	Espline TPtest	PCR (polA/tp47)	TP_negative6positive9	Lesion type
199	15	SNP	F	1	111115	Vervet monkey	*Chlorocebus pygerythrus*	-2.46187	34.80593	positive	NA	NA	CNA
200	16	SNP	M	1	121115	Vervet monkey	*Chlorocebus pygerythrus*	-2.29437	34.79967	positive	NA	NA	CNA
201	17	SNP	M	1	121115	Vervet monkey	*Chlorocebus pygerythrus*	-2.29460	34.80023	positive	NA	NA	CNA
202	18	SNP	F	1	121115	Vervet monkey	*Chlorocebus pygerythrus*	-2.29463	34.79988	positive	NA	NA	CNA
203	34	SNP	F	1	211115	Vervet monkey	*Chlorocebus pygerythrus*	-2.19287	33.87375	positive	NA	NA	CNA
204	35	SNP	F	1	211115	Vervet monkey	*Chlorocebus pygerythrus*	-2.19273	33.87362	positive	NA	NA	CNA
205	40	SNP	M	1	231115	Vervet monkey	*Chlorocebus pygerythrus*	-2.08465	34.64327	positive	NA	NA	CNA
206	41	SNP	M	2	231115	Vervet monkey	*Chlorocebus pygerythrus*	-2.08370	34.64142	positive	positive	positive	AG
207	01	SNP	M	5	120815	Olive baboon	*Papio anubis*	-2.23364	34.21735	positive	positive	positive	AG
208	02	SNP	F	2	130815	Olive baboon	*Papio anubis*	-2.37784	34.82267	positive	positive	positive	AG
209	04	SNP	M	1	071115	Olive baboon	*Papio anubis*	-2.43187	34.85440	negative	NA	NA	CNA
210	05	SNP	M	1	081115	Olive baboon	*Papio anubis*	-2.38373	34.81860	positive	NA	NA	CNA
211	06	SNP	F	2	081115	Olive baboon	*Papio anubis*	-2.38395	34.81907	positive	positive	positive	AG
212	07	SNP	M	5	081115	Olive baboon	*Papio anubis*	-2.48623	34.83500	positive	positive	positive	AG
213	08	SNP	M	1	091115	Olive baboon	*Papio anubis*	-2.49063	34.84278	negative	NA	NA	CNA
214	09	SNP	F	1	101115	Olive baboon	*Papio anubis*	-2.28761	34.68870	positive	NA	NA	CNA
215	10	SNP	F	1	101115	Olive baboon	*Papio anubis*	-2.27533	34.55577	negative	NA	NA	CNA
216	11	SNP	F	6	101115	Olive baboon	*Papio anubis*	-2.26867	34.66942	positive	NA	NA	OF
217	12	SNP	F	1	101115	Olive baboon	*Papio anubis*	-2.27402	34.67255	negative	NA	NA	CNA
218	13	SNP	F	1	111115	Olive baboon	*Papio anubis*	-2.46907	34.80675	negative	NA	NA	CNA
219	19	SNP	M	1	131115	Olive baboon	*Papio anubis*	-2.32102	34.84388	positive	NA	NA	CNA
220	20	SNP	M	1	131115	Olive baboon	*Papio anubis*	-2.32102	34.84367	positive	negative	NA	CNA
221	21	SNP	F	1	151115	Olive baboon	*Papio anubis*	-2.29965	34.78592	negative	NA	NA	CNA
222	22	SNP	M	1	151115	Olive baboon	*Papio anubis*	-2.29587	34.78653	negative	NA	NA	CNA
223	23	SNP	F	1	151115	Olive baboon	*Papio anubis*	-2.32980	34.84527	positive	NA	NA	CNA
224	24	SNP	M	5	151115	Olive baboon	*Papio anubis*	-2.33002	34.84545	positive	positive	positive	AG
225	25	SNP	F	1	161115	Olive baboon	*Papio anubis*	-2.08357	34.63790	negative	NA	NA	CNA
226	26	SNP	M	1	181115	Olive baboon	*Papio anubis*	-2.33135	34.84513	positive	NA	NA	CNA
227	27	SNP	M	1	191115	Olive baboon	*Papio anubis*	-2.19595	34.26968	positive	NA	NA	CNA
228	28	SNP	M	1	191115	Olive baboon	*Papio anubis*	-2.18507	34.23875	positive	NA	NA	CNA
229	29	SNP	F	2	191115	Olive baboon	*Papio anubis*	-2.18055	34.22652	positive	positive	positive	AG
230	30	SNP	M	1	191115	Olive baboon	*Papio anubis*	-2.18030	34.21208	positive	NA	NA	CNA
231	31	SNP	M	1	201115	Olive baboon	*Papio anubis*	-2.18847	34.11523	positive	NA	NA	CNA
232	32	SNP	F	1	201115	Olive baboon	*Papio anubis*	-2.18657	34.10915	positive	NA	NA	CNA
233	33	SNP	F	1	201115	Olive baboon	*Papio anubis*	-2.18312	34.10040	positive	NA	NA	CNA
234	36	SNP	F	1	211115	Olive baboon	*Papio anubis*	-2.19403	33.87282	negative	NA	NA	CNA
235	37	SNP	F	1	221115	Olive baboon	*Papio anubis*	-2.19155	33.87475	positive	NA	NA	CNA
236	38	SNP	F	1	221115	Olive baboon	*Papio anubis*	-2.19858	33.86595	positive	NA	NA	CNA
237	39	SNP	M	1	221115	Olive baboon	*Papio anubis*	-2.19840	33.86620	positive	NA	NA	CNA
238	42	SNP	M	1	231115	Olive baboon	*Papio anubis*	-2.08582	34.63492	positive	NA	NA	CNA
239	08	TNP	M	1	241215	Vervet monkey	*Chlorocebus pygerythrus*	-3.82708	36.06458	negative	NA	NA	CNA
240	09	TNP	M	1	241215	Vervet monkey	*Chlorocebus pygerythrus*	-3.82728	36.06422	positive	NA	NA	CNA
241	10	TNP	M	1	241215	Vervet monkey	*Chlorocebus pygerythrus*	-3.82725	36.06432	positive	NA	NA	CNA
242	19	TNP	M	1	281215	Vervet monkey	*Chlorocebus pygerythrus*	-3.12472	35.95083	positive	NA	NA	CNA
243	20	TNP	F	1	281215	Vervet monkey	*Chlorocebus pygerythrus*	-3.12468	35.95115	negative	NA	NA	CNA
244	26	TNP	F	1	010116	Vervet monkey	*Chlorocebus pygerythrus*	-3.74535	35.97062	negative	NA	NA	CNA
245	01	TNP	F	1	231215	Olive baboon	*Papio anubis*	-3.77943	35.96525	positive	NA	NA	CNA
246	02	TNP	M	1	231215	Olive baboon	*Papio anubis*	-3.78003	35.96580	negative	NA	NA	CNA
247	03	TNP	M	1	231215	Olive baboon	*Papio anubis*	-3.78090	35.96625	negative	NA	NA	CNA
248	04	TNP	F	1	231215	Olive baboon	*Papio anubis*	-3.78172	35.96652	negative	NA	NA	CNA
249	05	TNP	F	11	241215	Olive baboon	*Papio anubis*	-3.88543	36.07542	positive	positive	positive	OF&AG
250	06	TNP	F	1	241215	Olive baboon	*Papio anubis*	-3.88506	36.07558	positive	NA	NA	CNA
251	07	TNP	M	1	241215	Olive baboon	*Papio anubis*	-3.88543	36.07542	positive	NA	NA	CNA
252	11	TNP	F	1	251215	Olive baboon	*Papio anubis*	-3.78705	36.04322	positive	NA	NA	CNA
253	12	TNP	M	9	251215	Olive baboon	*Papio anubis*	-3.78723	36.04405	positive	positive	positive	OF
254	13	TNP	M	1	251215	Olive baboon	*Papio anubis*	-3.78783	36.04460	positive	NA	NA	CNA
255	14	TNP	M	1	261215	Olive baboon	*Papio anubis*	-3.79307	35.95858	negative	NA	NA	CNA
256	15	TNP	F	2	261215	Olive baboon	*Papio anubis*	-3.79297	35.95868	positive	positive	positive	AG
257	16	TNP	F	1	271215	Olive baboon	*Papio anubis*	-3.76803	36.02092	positive	NA	NA	CNA
258	17	TNP	M	1	271215	Olive baboon	*Papio anubis*	-3.76815	36.02072	positive	NA	NA	CNA
259	18	TNP	F	1	271215	Olive baboon	*Papio anubis*	-3.76742	36.02215	negative	NA	NA	CNA
260	21	TNP	F	1	311215	Olive baboon	*Papio anubis*	-3.78778	36.04100	positive	NA	NA	CNA
261	22	TNP	F	1	311215	Olive baboon	*Papio anubis*	-3.78675	36.04115	positive	NA	NA	CNA
262	23	TNP	M	1	311215	Olive baboon	*Papio anubis*	-3.78770	36.04482	positive	NA	NA	CNA
263	24	TNP	F	1	010116	Olive baboon	*Papio anubis*	-3.81748	36.05160	positive	NA	NA	CNA
264	25	TNP	F	1	010116	Olive baboon	*Papio anubis*	-3.81863	36.05258	positive	NA	NA	CNA
265	06	UNP	M	1	230616	Blue monkey	*Cercopithecus mitis*	-7.84487	36.88357	negative	NA	NA	CNA

S/No.	ID No.	Geo location	Sex	Numerical ID code	Sampling date	Species (common name)	Species (scientific name)	Latidude	Longitude	Espline TP test	PCR (polA/tp47)	TP_negative&positive9	Lesion type
266	07	UNP	F	1	230616	Blue monkey	*Cercopithecus mitis*	-7.84493	36.88377	negative	NA	NA	CNA
267	10	UNP	M	1	020716	Blue monkey	*Cercopithecus mitis*	-7.84512	36.88407	negative	NA	NA	CNA
268	11	UNP	M	1	030716	Blue monkey	*Cercopithecus mitis*	-7.84480	36.88353	negative	NA	NA	CNA
269	08	UNP	M	2	010716	Vervet monkey	*Chlorocebus pygerythrus*	-7.84463	36.89220	positive	positive	positive	AG
270	01	UNP	M	1	220616	Yellow baboon	*Papio cynocephalus*	-7.85593	36.89015	negative	NA	NA	CNA
271	02	UNP	M	1	220616	Yellow baboon	*Papio cynocephalus*	-7.84488	36.88652	negative	NA	NA	CNA
272	03	UNP	F	1	220616	Yellow baboon	*Papio cynocephalus*	-7.84472	36.88388	negative	NA	NA	CNA
273	04	UNP	M	1	230616	Yellow baboon	*Papio cynocephalus*	-7.84347	36.88682	negative	NA	NA	CNA
274	05	UNP	F	1	230616	Yellow baboon	*Papio cynocephalus*	-7.84385	36.88650	negative	NA	NA	CNA
275	14	UNP	F	1	050716	Yellow baboon	*Papio cynocephalus*	-7.85745	36.89092	negative	NA	NA	CNA
276	15	UNP	M	1	050716	Yellow baboon	*Papio cynocephalus*	-7.85807	36.89122	negative	NA	NA	CNA
277	18	UNP	M	1	100317	Yellow baboon	*Papio cynocephalus*	-7.52151	36.60478	positive	NA	NA	CNA
278	19	UNP	M	1	100317	Yellow baboon	*Papio cynocephalus*	-7.52020	36.59877	negative	NA	NA	CNA
279	20	UNP	M	1	100317	Yellow baboon	*Papio cynocephalus*	-7.49790	36.56256	negative	NA	NA	CNA
280	21	UNP	M	1	110317	Yellow baboon	*Papio cynocephalus*	-7.52070	36.62044	negative	NA	NA	CNA
281	22	UNP	M	1	110317	Yellow baboon	*Papio cynocephalus*	-7.51679	36.62833	positive	NA	NA	CNA
282	23	UNP	F	1	120317	Yellow baboon	*Papio cynocephalus*	-7.49594	36.56725	negative	NA	NA	CNA
283	24	UNP	F	1	150317	Yellow baboon	*Papio cynocephalus*	-7.81512	36.89523	negative	NA	NA	CNA
284	25	UNP	F	1	150317	Yellow baboon	*Papio cynocephalus*	-7.84805	36.88613	negative	NA	NA	CNA
285	26	UNP	M	1	150317	Yellow baboon	*Papio cynocephalus*	-7.84988	36.88511	negative	NA	NA	CNA
286	27	UNP	M	1	160317	Yellow baboon	*Papio cynocephalus*	-7.80575	36.89683	negative	NA	NA	CNA
287	09	UNP	M	1	010716	Udzungwa red colobus	*Piliocolobus gordonorum*	-7.84475	36.88543	negative	NA	NA	CNA
288	12	UNP	F	1	030716	Udzungwa red colobus	*Piliocolobus gordonorum*	-7.84467	36.88382	negative	NA	NA	CNA
289	13	UNP	M	1	030716	Udzungwa red colobus	*Piliocolobus gordonorum*	-7.84473	36.88413	negative	NA	NA	CNA

CHAPTER FOUR

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Strain diversity of *Treponema pallidum* subsp. *pertenue* suggests rare interspecies transmission in African nonhuman primates

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In our most recent study, we found that in Tanzania infection with *Treponema pallidum* (*TP*) subsp. *pertenue* (*TPE*) is present in four different monkey species. In order to gain information on the diversity and epidemiological spread of the infection in Tanzanian nonhuman primates (NHP), we identified two suitable candidate genes for multi-locus sequence typing (MLST). We demonstrate the functionality of the MLST system in invasively and non-invasively collected samples. While we were not able to demonstrate frequent interspecies transmission of *TPE* in Tanzanian monkeys, our results show a clustering of *TPE* strains according to geography and not host species, which is suggestive for rare transmission events between different NHP species. In addition to the geographic stability, we describe the relative temporal stability of the strains infecting NHPs and identified multi-strain infection. Differences between *TPE* strains of NHP and human origin are highlighted. Our results show that antibiotic resistance does not occur in Tanzanian *TPE* strains of NHP origin.

Nonhuman primates (NHPs) in Africa are naturally infected with *Treponema pallidum* subsp. *pertenue* (*TPE*)[1], the bacterium causing human yaws. In our most recent study, we found that in Tanzania infection with *TPE* is present in four different NHP species (olive baboon (*Papio anubis*), yellow baboon (*Papio cynocephalus*), vervet monkey (*Chlorocebus pygerythrus*), and blue monkey (*Cercopithecus mitis*))[2]. Moreover, we showed that infection is geographically widespread within Tanzania. Although we confirmed infection by serology and PCR, the data were insufficient to describe the epidemiology of the disease. Further insights into the inter- and intraspecies spread of the *TPE* bacterium in Tanzanian NHPs will contribute to our understanding of transmission pathways and pathogen maintenance, which are crucial elements for the identification of a functional disease reservoir[3].

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Locus[$]	Length	Protein function	No. of variable sites	SNVs frequency/kbp
TPESAMD_0136	1,412	FIBRONECTIN-BINDING protein	41	29.04
TPESAMD_0548	1,298	FadL ortholog, outer membrane protein	36	27.73
TPESAMD_0858	1,229	FadL ortholog	33	26.85
TPESAMD_0488	2,537	METHYL-accepting chemotaxis protein	31	12.22
TPESAMD_0865	1,445	FadL ortholog	26	17.99
TPESAMD_0326	2,502	BamA	22	8.40

Table 1. Genes with the highest SNVs frequency per kbp containing 22 and more SNVs among samples listed in Table S1. [$]Anotation and length of the genes were identified according to the *TPE* reference genome Samoa D (GenBank accession number CP002374.1). Protein predictions by Brinkman *et al.*[35] and Radolf and Kumar[36].

The chance that NHPs infected with *TPE* are a potential source for human infection has been discussed for tropical Africa[4]. However, naturally occurring transmission from NHPs to humans and *vice versa* has not been confirmed by current data, although phylogenetic analyses of whole genome sequences from *TPE*s of human and NHP origin suggest a rapid initial radiation of the ancestor of *TPE* across the different primate taxa, including humans[1].

Molecular typing is used to accurately distinguish between different strains of *T. pallidum* (*TP*) for epidemiological and surveillance analysis. The method has been extensively applied to the syphilis–causing bacterium (subsp. *pallidum*, *TPA*)[5] where it is used to describe its spatial, e.g.[6–9], and temporal, e.g.[10], subtype composition. Two recent studies suggested new typing systems for human yaws[11,12]. It was unclear though, whether the existing molecular typing systems for *TPA* or *TPE* of human origin can be applied to *TP* strains originating from NHPs.

In the current study, we identified suitable candidate genes for multi-locus sequence typing (MLST) in *TP* samples of NHP origin and investigated strain diversity of the NHP infecting strains in Tanzania. We hypothesized that interspecies transmission in NHPs is ongoing. Moreover, we show that our typing system can be applied to samples from other regions of Africa and to analyze *TP* in non-invasively collected fecal samples.

Materials and Methods

Ethical statement. No animals were handled specifically for this study. The ethical statement for the Tanzanian NHP samples has been published elsewhere[2,13]. Lesion swabs from Ethiopian grivet monkeys (*Chlorocebus aethiops*) were taken as part of a research investigation conducted by AA under the Ethiopian Wildlife Conservation Authority reference number 15ET-0000-BS-01. Noninvasively collected fecal samples of western lowland gorillas (*Gorilla gorilla gorilla*) from the southern part of Odzala-Kokoua National Park (OKNP) originate from a collaboration signed under a MoU between the Foundation Odzala-Kokoua, the German Primate Center, and the Institute of Vertebrate Biology, Czech Academy of Sciences in November 2017.

Design of the multi-locus sequence typing system. In order to identify most suitable candidate genes for MLST in *TPE* strains of NHP origin, we used 23 available complete and draft genome sequences of *TPE* from both human and NHPs from Africa and the Pacific regions (Table S1). Several criteria were applied to obtain most suitable gene loci for *TPE* MLST. First, we identified the most variable genes with accumulated single nucleotide variants (SNVs) in short DNA fragments (genes containing the highest SNVs frequency per kbp) and, at the same time, with potential ability to distinguish all strains used for this analysis (containing 22 and more variable sites; Table S1). We identified six candidate genes (Table 1) and compared the resolution power of phylogenetic trees based on genome-wide data and phylogenetic trees based on sequences of individual genes.

With this approach, we propose a new MLST scheme for *TPE* strains of NHP origin, based on sequencing of two variable loci (*TP0548* and *TP0488*). The typing scheme is able to reveal 70% of whole genome resolution. Further details on the identification of most variable genes, resolution power, and the selection of most suitable typing loci are provided in the Technical Appendix.

Samples included into the study and DNA extraction. Our study used *TP* positive DNA samples from 85 NHPs of six different species and three African countries (Tables 2 and S3). The samples originated from previously published[2,13] and ongoing research investigations. The different methods of DNA extraction are presented in the Technical Appendix.

DNA target enrichment. Before MLST, DNA extracted from fecal samples was enriched for bacterial DNA using the Looxter Enrichment Kit (Analytik Jena, Jena, Germany) following the manufacturer's protocol.

Polymerase chain reactions. *Multi-Locus Sequence Typing system. TP0548*: Amplification of a fragment of the *TP0548* gene was achieved using a nested PCR. The two-step PCR amplified a 1,065-bp long fragment of the target gene. Amplification and sequencing primers were used as reported elsewhere[14]. Briefly, the 50-µl reaction volume comprised 25 µl of the 2x Universe buffer (Universe High Fidelity Hot Start DNA Polymerase Kit, Biotool, Munich, Germany), 17 µl RNAase free water, 2 µl of each 10 µM primer, 1 µl DNA polymerase (1 U/µl), 1 µl of the dNTP mix (10 mM each), and 2 µl template DNA, independent of DNA concentration. Amplification was performed in a SensoQuest Labcycler using the following thermocycler conditions: pre-denaturation at 95 °C for 3 min, followed by 40 and 30 cycles, respectively, each with 95 °C for 15 sec, 48 °C for 15 sec, and 72 °C for 30 sec. Each of the PCR runs ended with a post-extension step at 72 °C for 5 min.

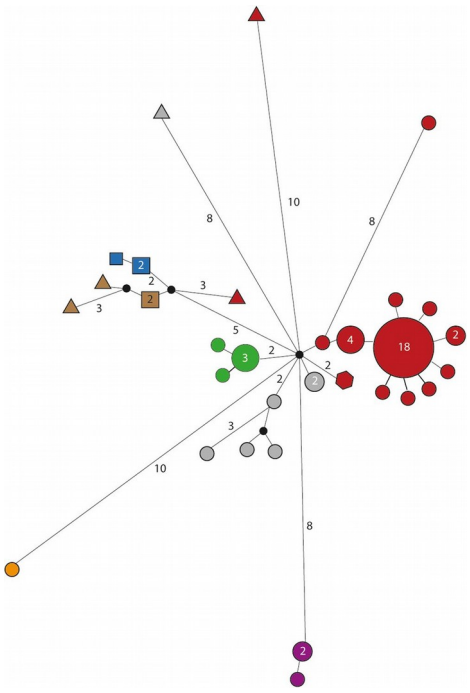

Figure 1. Median-joining network using 1,773 bp – long concatemer of *TP0488* and *TP0548* loci from 57 Tanzanian NHPs samples. The number of mutations, when >1, is given close to branches. Inferred allelic variants (median vectors) are shown as small black connecting circles. If contiguous, indels were considered as a single event only. The number of individuals, when >1, is shown inside the circles and are dependent on circle size. Species trait is given in the geometric form: circle = *Papio anubis* (n = 46); squares = *Papio cynocephalus* (n = 5); triangles = *Chlorocebus pygerythrus* (n = 5); hexagon = *Cercopithecus mitis* (n = 1). Sample location trait is given by the color code: blue = UG (n = 3); orange = TN (n = 1); brown = Ruaha National Park (n = 4); red = Lake Manyara National Park (n = 34); grey = Serengeti National Park (n = 7); green = Ngorongoro Conservation Area (n = 5); violet = Gombe National Park (n = 3).

NHP samples where a high number of samples came from one geographic location. However, we used the complete concatenated sequence alignment, including the data obtained from the grivet monkey and gorilla samples, to construct ML (Fig. 2) and MP (Fig. S6) trees. For the latter, gaps were coded as fifth character. NHP strains included in this study clustered with human yaws-causing strains and were clearly separated from the *TEN* strain Bosnia A as well as the human syphilis-causing *TPA* strains (Figs 2 and S7). Within the *TPE* clade, bootstrap support was mostly weak (<80%) with some exceptions. One of these notable exceptions was the separation of the grivet monkey infecting strain from Ethiopia and the strain that was generated from a gorilla fecal sample. Sequences of both strains (14AWM2051017 and 3DZAKM13280917) differed in only one nucleotide position and always clustered together (Figs 2 and S6). Overall, we found a geographic clustering of *TPE* strains, instead of clustering by host species. None of the samples from the Tanzanian NHPs (n = 72/76; four samples did not generate a PCR product) has been tested positive for the mutations in the 23 S ribosomal RNA genes that code for macrolide resistance. The grivet monkey and the gorilla samples were not tested for microbial resistance.

Sequences obtained from the *TP0619* gene were identical across all NHP species and sampling sites, including the grivet monkey and the gorilla samples. A representative sequence obtained from a vervet monkey sampled at Katavi National Park (4KNF2121016) was published previously under the GenBank accession number MF754122[2].

In general, all NHP-derived sequences of the *TP0548* locus including the grivet monkey and the gorilla sequence, contained one section of the sequence where most of the nucleotide variation is found (Fig. S1). This distinguishes them not only from human syphilis- (*TPA*) and bejel-causing (*TEN*) strains but also from *TPE* strains of human origin, where there are three and two variable regions, respectively. Sequences of the *TP0548*

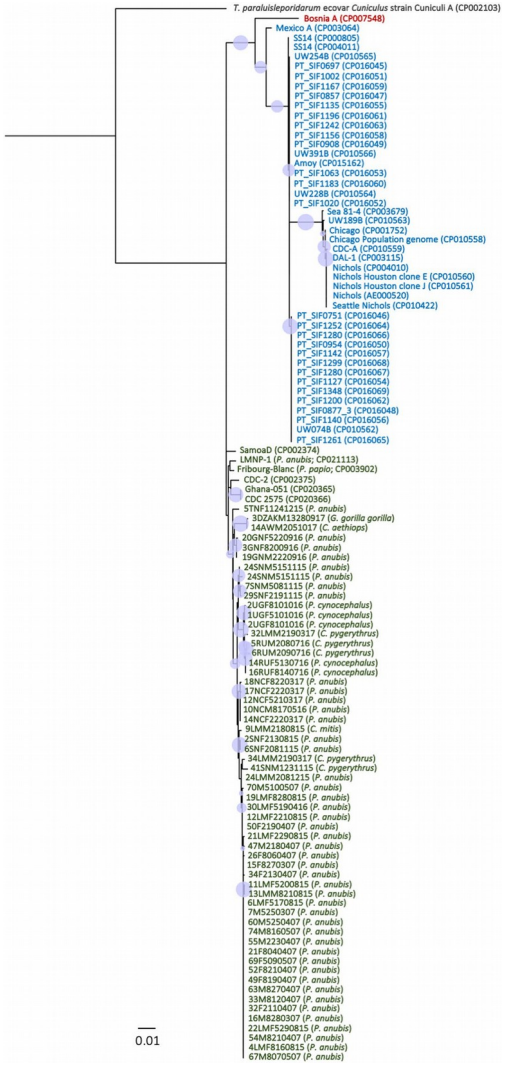

Figure 2. Rooted ML tree based on the concatenated sequences used for MLST (*TP0548* and *TP0488*). The tree is based on 1,773 nts and 1,000 bootstrap replicates. Bootstrap values from 80–100% are highlighted as light blue circles of respective size. NHP species and/or GenBank accession numbers of published strains are provided in parentheses following the name of the strain. In all cases were the species is not mentioned, sequences are from *TP* of human origin. Blue = subsp. *pallidum*, green = subsp. *pertenue*, red = subsp. *endemicum*. The pathogen causing rabbit syphilis, *Treponema paraluisleporidarum* ecovar *Cuniculus* strain Cuniculi A, is used as an outgroup. The bar refers to substitutions per site.

and *TP0488* loci showed comprehensive variability within and across the different sampling locations as well as between the different NHP species in Tanzania. Corresponding ML and MP trees each constructed for *TP0548* (Figs S2 and S3) and *TP0488* (Figs S4 and S5) were similar in topology. We note here that sample size was low

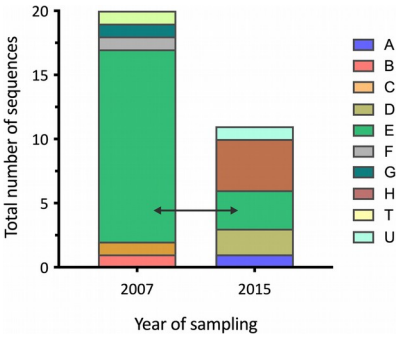

Figure 3. Temporal strain composition for samples collected from olive baboons at Lake Manyara National Park. The different colors indicate different genotypes. Sampling of olive baboons at LMNP was conducted in 2007 and 2015. The strain "E" (light green; e.g., 4LMF8160815) was the dominating strain in 2007 and was still present in baboons sampled eight years later (2015).

for some of the species (e.g., blue monkey) and that we included here only the Tanzanian samples to match the network analysis (Fig. 1).

Since the largest number of samples was taken at Lake Manyara National Park (LMNP) and samples were collected in 2007, 2015, and 2017, respectively, we were able to plot the temporal strain composition for this specific sampling location. Figure 3 illustrates the temporal strain composition for all samples that originate from olive baboons. Using a sequence alignment and base-by-base comparison, we found that in 2007 there were six strains, with a dominating strain "E" (e.g., 4LMF8160815). Samples from olive baboons at LMNP taken eight years later reconfirmed the existence of this strain. The temporal stability of strains was further supported by strain "P" (e.g., 10NCM8170516) that was collected from two olive baboons at the Ngorongoro Conservation Area in 2015 and one olive baboon in 2016 (Table S4).

Discussion

Frequent transmission of *TPE* strains across the different NHP species would likely result in a higher number of shared and identical sequences in different species at the same geographic site. Although we found no identical strain sequences in different primate species at one site (Table S4), we still observed a geographic clustering of (closely related) *TPE* strain sequences and not a clustering according to NHP host species (Fig. 1). This suggests that interspecies transmission occurs, albeit rarely. That we found no identical strain sequences in different primate species could be due to the small sample size for some of the investigated NHP species, but generally argues against frequent transmission. This gains further support by our finding that the strains of NHP origin are relatively stable over time as we show for LMNP (Fig. 3, Table S4). This is consistent with what we know from human infecting *TPA* strains[21–23]. The 2007 dominating strain "E" (e.g., 4LMF8160815), which was isolated from olive baboons was still present in the infected baboon population in 2015. Although we do not see a strict geographic pattern when the two target genes were analyzed individually (Figs S2–S5), when examining the concatenated alignment (median-joining network (Fig. 1) and the ML tree (Fig. 2)), geographic strain clustering can be observed. This is another indicator for the relative temporal and geographic stability of the strains that infect NHPs. The recently estimated low mutation rate in human *TPE* strains[22] coincides with the genetic stability observed among NHP *TPE* strains analyzed in this study. Yet, feasible interspecies transmission routes for *TP* exist[3] and have been discussed for flies[24] and were proven for sexual intercourse between different NHP species based on host genetic data[25].

Despite the differences in the number of variable sites at the *TP0548* locus (Fig. S1), we see a close association of NHP and human infecting *TPE* strains. This was expected, since the genome of *TPE* LMNP-1 strain (GenBank accession number CP021113), which was obtained from an olive baboon at LMNP in 2007, was found to be closely related to human yaws causing strains similar to all other *TPE* strains of NHP origin[1].

The absence of antibiotic resistance to azithromycin in all tested *TP* strains from NHPs in Tanzania is a positive sign and is probably related to the absence of treatment of infected monkeys. Currently only Gombe National Park has a history of treating infected baboons with antimicrobials[26]. In human yaws, it has been shown that after a single treatment round with antibiotic macrolides, resistance emerges[27] even though the *de novo* emergence of such mutations is lower than 10^{-3} per treated patient[28]. The risk of emerging antimicrobial resistance is of major concern for human infection and would also draw major implications for the conservation of endangered NHP species such as gorillas (*Gorilla gorilla*). Similar to human yaws elimination[27], responsible treatment of infected NHP populations requires resistance monitoring and possible ring-fencing with effective alternative antimicrobials.

The identification of two different strains obtained from independent PCRs in three different NHPs (*TP0548*: 11LMF8190815 (*P. anubis*; 15-bp indel); *TP0488*: 2UGF8101016 (*P. cynocephalus*; one SNV) and 24SNM5151115

(*P. anubis*; three SNVs); Table S4) supports the concept of absence of cross immunity between different *TP* strains[29] and boosts evidence for recombination events found in the *TP* bacterium[30–32] under natural conditions.

The initial CDC typing system for *TPA* made use of the number of 60-bp repeats found in the acidic repeat protein (*arp* (*TP0433*)) gene in combination with differences found in the *tpr* subfamily II genes (*tprE* (*TP0313*), *tprG* (*TP0317*), and *tprJ* (*TP0621*)[9]. A subsequently introduced enhanced typing system included a portion of the *TP0548* locus[33]. While the enhanced typing system has also been used for the typing of human *TPE* strains[12], it did not overcome the difficulties associated with amplification of 60-bp repeats of the *arp* gene or the uncertainties associated with amplification of three different *tpr*-subfamily II genes in one single assay, followed by subsequent restriction enzyme analysis[11]. A recently published alternative method for MLST included the widely used *TP0548* locus but also two additional loci located in the *TP0136* and *TP0326* genes[11]. We took these loci into account, but in our analysis, it became evident that the *TP0136* locus in the Tanzanian *TP* strains of NHP origin was highly conserved. A representative sequence is accessible under GenBank accession number CP021113.1 (nt158,275-159,195). Whether this is a characteristic of NHP infecting strains or a spatial property of strains originating from Tanzania is unknown. It underlines, however, that a globally applied strain typing system for *TPE* requires a comprehensive database of high-quality genomes obtained from larger numbers of clinical samples from yaws endemic areas.

We identified several gene loci as suitable candidates to be used in *TPE* strains of human and NHP origin. The two gene targets that we selected for MLST (*TP0548* and *TP0488*) originated from a number of suitable candidates (Table 2) and selection was based on best PCR performance in the clinical NHP samples that were included in this study. We neither aimed for the design of a *TPE* typing system that can be used in a clinical environment for human infection, nor did we anticipate a typing system that is suitable for a global approach. Amplification of relatively long sequence parts (e.g., *TP0548* enhanced typing system determines 84 bp[33] vs. 1,065 bp determined in this study) was therefore not considered an issue. Both loci that were used for the typing, *TP0548* and *TP0488*, are reported to show signs of recombination in human syphilis causing *TPA* strains[30–32]. This, however, was not considered a limitation since our MLST typing system was designed to describe strain variability within a given population of NHPs and is not used to describe a detailed geophylogeny of *TP*. While the *TP0619* sequence, which codes for a protein family of Domains of Unknown Function (DUF)2715 and which appear to be restricted to *TP*, is not part of our typing system, it is useful to support the difference of the *TP* strains of NHP origin analyzed in this study from human syphilis-causing *TPA* strains. It is currently unclear if wild NHPs are also naturally infected with *TPA* strains.

Invasive sampling of NHPs in the wild is generally associated with challenges in terms of ethics and logistics. As a consequence, it is easier to obtain a greater number of non-invasively collected fecal samples than samples that originate from invasively sampled individuals. Moreover, prospective epidemiological studies would benefit from sampling regimes that allow the screening and subsequent typing of *TP* strains in non-invasively collected samples from a greater geographic area. For this reason, we tested our newly established MLST system in non-invasively collected fecal samples from gorillas at Odzala-Kokoua National Park in the Republic of the Congo, a place where gorillas with ulcerative skin lesions have been frequently sighted[34] (Fig. S7). We were able to successfully strain type the gorilla samples and could show that the strain from which we obtained all three target sequences, clusters with a strain isolated from a grivet monkey in Ethiopia. The reason for the close association of the strain of the grivet monkey and the gorilla origin is currently unclear and answering this question requires intensified sampling in the respective geographic areas and in-between to obtain more sequence data.

Conclusion

The high number of *TP* infected NHPs in Africa, the different species that have been confirmed as hosts[2,4], as well as the recently documented close genetic and functional similarity of NHP and human infecting *TPE*[1] requires epidemiological data for a better understanding on how the infection is maintained in primate populations and whether or not it is transmitted to humans. Our study provides an important contribution to answer the question on interspecies transmission in primate infecting *TP*, although further sampling is needed to increase confidence in the results. However, with our data we were able to show that interspecies transmission in Tanzanian monkeys is likely although rare. As humans are primates, the most important question to answer in future studies is, whether or not *TPE* strains of NHP origin transmit to humans and/or *vice versa*. The interspecies transmission of *TPE* in nonhuman primates, is not necessarily predictive for spillovers to humans.

Data Availability

GenBank accession numbers for the sequences generated in this study can be found in Table S5.

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Author Contributions

The study was designed by I.S.C., C.R., D.S. and S.K. Design of the MLST system was done by C.R., L.G., J.O., D.S. and S.K. Laboratory work took place at Sokoine University of Agriculture and the German Primate Center and was performed by I.S.C., C.R., L.H.W., S.L. and S.K. Data were analyzed by I.S.C., C.R., D.S. and S.K. All authors (I.S.C., C.R., A.A., T.B., D.A.C., L.G., L.H.W., R.R.K., J.D.K., S.L., U.M., J.O., K.J.P., A.P., F.A.S., D.S. and S.K.) contributed to the manuscript preparation.

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Technical appendix

Strain diversity of *Treponema pallidum* subsp. *pertenue* suggests rare interspecies transmission in African nonhuman primates

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Design of the Multi-Locus Sequence Typing system

Identification of most variable genes in TPE

We identified six candidate genes with the SNVs frequency per kbp ranging from 8-29, which code outer membrane proteins or hypothetical proteins (*TP0136*, *TP0326*, *TP0488*, *TP0548*, *TP0858*, *TP0865*; Table 1). Most of the loci were identified as putative intra- or inter-strain recombination genes (*TP0136*, *TP0326*, *TP0488*, *TP548* and *TP0865*)[30,32,37-38].

Resolution power of genome-wide data and individual loci

Genome-wide maximum-likelihood (ML) trees of 23 available complete and draft genome sequences were based on 1,207 variable sites and allowed to distinguish 22 haplotypes (data not showed). Given the fact that strains CDC 2575 and Ghana-051 are completely identical[20], the genome-wide tree had 100% resolution of whole genome sequences. Among six candidate loci, the highest resolution power was observed in *TP0488* (70%) followed by *TP0326*,

TP0548, *TP0858* (all 57%), *TP0136* (44%) and *TP0865* (31.8%). Interestingly, the concatenated sequences of all candidate loci did not reveal higher resolution then the resolution observed in the *TP0488* gene (70%).

Identification of the most suitable typing loci for TPE MLST among and within the candidate genes

Since the amplification efficiency of *Treponema* DNA from clinical samples has been shown to be dependent on the length of the PCR products[10,39], we selected loci with the highest occurrence of variable sites accumulated in as short DNA regions as possible (Table S2). Furthermore, we preferred loci with the highest percentage of genome-wide resolution and last, we selected loci that were able to clearly distinguish treponematoses caused by *TPE* from the *TPA*/*TEN* infections (Table S2).

DNA extraction

DNA extraction for tissue samples was performed as described elsewhere[2]. Swab materials followed the same protocol as described for the skin tissues using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) with some modifications. Briefly, each swab was digested in 450 µl custom-made lysis buffer (10 mM Tris [pH 8.0], 0.1 M EDTA [pH 8.0], and 0.5% sodium dodecyl sulfate), the same buffer in which the swab was collected. In addition, the remaining lysis buffer was also digested. In both reactions, care was taken to keep the ratio of buffer and proteinase K as recommended by the manufacturer. The samples were digested overnight at 56°C and 900 rpm (Thermomixer Comfort, Eppendorf, Hamburg, Germany). On the next day, reaction tubes that contained swabs were placed on top of a new PCR clean reaction tube of 2.0 ml volume. A sterile 20G needle was used to penetrate the bottom of the reaction tube to allow for subsequent separation of DNA containing fluid from the swab through vigorous centrifugation at 6,000 xg for 5 min at room temperature. All subsequent steps followed the

manufactures protocol. The DNA was eluted twice with 100 µL AE buffer and was further purified using glycogen precipitation according to the protocol published in Knauf et al.[24].

Ethanol (98%)-preserved fecal samples from western lowland gorillas (*Gorilla gorilla gorilla*) were extracted using the First DNA All-tissue extraction kit (Gen-ial, Troisdorf, Germany). Briefly, the procedure followed the manufacturer's guidance with some minor modifications. Feces were dried from ethanol overnight and subsequently resolved in 1 ml of the kit's containing lysis buffers #1 and 100 µl of lysis buffer #2. Samples where then incubated with 20 µl proteinase K at 65°C and 1000 rpm (Thermomixer Comfort, Eppendorf, Hamburg, Germany) for 50 min. All subsequent steps followed those of the manufacture's protocol.

Table S1. *TPE* genomes used to identify most suitable gene candidates for the MLST design. [§] In order to determine the whole genome sequence, the *TPE* strain was multiplied in experimental animals prior to the NGS. [§] Culture-independent enrichments (hybridization captures) were used prior to the NGS in order to separate the *TP* genetic material from the host DNA. Compl.=complete

Strains ID	Genome	Source	Host	Year of isolation	Geographic area	References
CDC-1	Compl.	Rabbit inoculation[§]	Human	1980	Ghana	unpublished
CDC-2	Compl.	Rabbit inoculation[§]	Human	1980	Ghana	40
Samoa D	Compl.	Rabbit inoculation[§]	Human	1953	Western Samoa	40
Gauthier	Compl.	Rabbit inoculation[§]	Human	1960	Congo	40
Fribourg-Blanc	Compl.	Rabbit inoculation[§]	NHPs	1966	Ghana	41
CDC 2575	Compl.	Rabbit inoculation[§]	Human	1980	Ghana	21
Ghana-051	Compl.	Rabbit inoculation[§]	Human	1988	Ghana	21
Sei Geringging K403	Compl.	Rabbit inoculation[§]	Human	1990	Indonesia	42
Kampung Dalan K363	Compl.	Rabbit inoculation[§]	Human	1990	Indonesia	42
LMNP-1	Compl.	Clinical[§]	NHPs	2007	Tanzania	1
LMNP-2	Draft	Clinical[§]	NHPs	2007	Tanzania	1
Gambia-1	Draft	Clinical[§]	NHPs	unknown	Gambia	1
Gambia-2	Draft	Clinical[§]	NHPs	unknown	Gambia	1
Senegal NKNP-1	Draft	Clinical[§]	NHPs	unknown	Senegal	1
Senegal NKNP-2	Draft	Clinical[§]	NHPs	unknown	Senegal	1
Cote d'Ivoire TaiNP-1	Draft	Clinical[§]	NHP	unknown	Ivory Coast	1
Cote d'Ivoire TaiNP-2	Draft	Clinical[§]	NHP	unknown	Ivory Coast	1

ERR1470330	Draft	Clinical§	Human	2013	Solomon Islands	43
ERR1470331	Draft	Clinical§	Human	2013	Solomon Islands	43
ERR1470334	Draft	Clinical§	Human	2013	Solomon Islands	43
ERR1470338	Draft	Clinical§	Human	2013	Solomon Islands	43
ERR1470343	Draft	Clinical§	Human	2013	Solomon Islands	43
ERR1470344	Draft	Clinical§	Human	2013	Solomon Islands	43

68 **Table S2: Characteristics of candidate genes for *TPE* typing.** [$] According to the yaws
69 reference genome Samoa D (GenBank accession number CP002374.1). [§] The genomes listed
70 in Table 1 including representatives of *TPE* strains, representative of TEN (Bosnia A,
71 GenBank accession number CP007548.1) and representatives of *TPA* (Nichols, GenBank
72 accession number CP004010.2; SS14, GenBank accession number CP004011.1; Mexico,
73 GenBank accession number CP003064.1) were used to test whether the corresponding
74 sequences can be used for subspecies classification (*TPA*/*TPE*/*TEN*).

Gene[$]	Variable region length (bp)	Variable region coordinates[$]	% of genome-wide data resolution	Differen-tiating TPE from TPA/TEN[§]	Differen-tiating TPA/TPE/TEN[§]
TP0488	782	522,942 – 523,723	70.0	Yes	No
TP0548	755	593,318 – 594,072	57.0	No	No
TP0858	824	936,118 – 936,941	57.0	Yes	Yes
TP0326	2,086	346,066 – 348,151	57.0	Yes	No
TP0136	910	157,823 – 158,733	44.0	Yes	Yes
TP0865	897	945,224 – 946,121	31.8	Yes	Yes

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78 **Table S3. Summary of *TP* sequence data of NHP origin included into this study.** *multi-
79 strain infection present, NP=National Park, CA=Conservation Area, TZ=Tanzania,
80 ET=Ethiopia, RC=Republic of the Congo. Details on NHP species composition can be found
81 in Table S5.

Country	Sample location	n NHPs	n *TP0619* sequences	n *TP0548* sequences	n *TP0488* sequences	n concatenated sequences
TZ	Gombe NP	3	1	3	3	3
TZ	Lake Manyara NP	46	41	44*	37	33
TZ	Katavi NP	1	1	0	1	0
TZ	Mahale NP	1	1	1	0	0
TZ	Mikumi NP	1	0	1	0	0
TZ	Ngorongoro CA	9	9	9	5	5
TZ	Ruaha NP	6	4	5	5	4
TZ	Serengeti NP	7	5	7	8*	8
TZ	Tarangire NP	2	1	2	1	1
TZ	Issa Valley	2	2	2	3*	3
TZ	Udzungwa NP	1	1	1	0	0
ET	Awash NP	2	1	2	1	1
RC	Odzala-Kokoua NP	4	4	1	4	1
	Total	85	71	78	67	59

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Table S4. Strains diversity and year of sampling based on the concatenated sequences used for MLST (*TP0548* and *TP0488*) in NHP infecting *TP*. Strain classification letters (uppercase) shall not be confused with lower case letters that are used in the enhanced typing system in human syphilis and yaws-causing strains. The letters used here are only used to visualize and discuss the differences in *TP* strains of NHP origin.

Strain Typ	NHP sample ID	Sampling Area	Sampling Year	Species
A	21LMF2290815	LMNP	2015	*Papio anubis*
B	26F8060407	LMNP	2007	*Papio anubis*
C	47M2180407	LMNP	2007	*Papio anubis*
D	11LMF5200815	LMNP	2015	*Papio anubis*
D	13LMM8210815	LMNP	2015	*Papio anubis*
E	4LMF8160815	LMNP	2015	*Papio anubis*
E	6LMF5170815	LMNP	2015	*Papio anubis*
E	7F5250307	LMNP	2007	*Papio anubis*
E	15F8270307	LMNP	2007	*Papio anubis*
E	16M8280307	LMNP	2007	*Papio anubis*
E	21F8040407	LMNP	2007	*Papio anubis*
E	22LMF5290815	LMNP	2015	*Papio anubis*
E	32F2110407	LMNP	2007	*Papio anubis*
E	33M8120407	LMNP	2007	*Papio anubis*
E	49F8190407	LMNP	2007	*Papio anubis*
E	52F8210407	LMNP	2007	*Papio anubis*
E	54M8210407	LMNP	2007	*Papio anubis*
E	55M2230407	LMNP	2007	*Papio anubis*
E	60M5250407	LMNP	2007	*Papio anubis*
E	63M8270407	LMNP	2007	*Papio anubis*
E	67M8000507	LMNP	2007	*Papio anubis*
E	69F5090507	LMNP	2007	*Papio anubis*
E	74M8160507	LMNP	2007	*Papio anubis*
F	34F2130407	LMNP	2007	*Papio anubis*
G	70M5100507	LMNP	2007	*Papio anubis*
H	12LMF2210815	LMNP	2015	*Papio anubis*
H	19LMF8280815	LMNP	2015	*Papio anubis*
H	30LMF5190416	LMNP	2015	*Papio anubis*
H	50F2190407	LMNP	2015	*Papio anubis*
I	5TNF11241215	TNP	2015	*Papio anubis*
J	24SNM5151115	SNP	2015	*Papio anubis*
K	29SNF2191115	SNP	2015	*Papio anubis*
L	7SNM5081115	SNP	2015	*Papio anubis*

M	24SNM5151115	SNP	2015	*Papio anubis*
N	20GNF5220916	GNP	2016	*Papio anubis*
O	19GNM2220916	GNP	2016	*Papio anubis*
O	3GNF8200916	GNP	2016	*Papio anubis*
P	10NCM8170516	NCA	2016	*Papio anubis*
P	12NCF5210317	NCA	2017	*Papio anubis*
P	14NCM5210317	NCA	2017	*Papio anubis*
Q	17NCF2220317	NCA	2017	*Papio anubis*
R	18NCF8220317	NCA	2017	*Papio anubis*
S	2SNF2130815	SNP	2015	*Papio anubis*
S	6SNF2081115	SNP	2015	*Papio anubis*
T	LMNP-1	LMNP	2007	*Papio anubis*
U	24LMM2081215	LMNP	2015	*Papio anubis*
V	34LMM2190317	LMNP	2017	*Chlorocebus pygerythrus*
W	41SNM1231115	SNP	2015	*Chlorocebus pygerythrus*
X	9LMM2180815	LMNP	2015	*Cercopithecus mitis*
Y	5RUM2080716	RNP	2016	*Chlorocebus pygerythrus*
Z	14RUF5130716	RNP	2016	*Papio cynocephalus*
Z	16RUF8140716	RNP	2016	*Papio cynocephalus*
AA	6RUM2090716	RNP	2016	*Chlorocebus pygerythrus*
AB	32LMM2190317	LMNP	2017	*Chlorocebus pygerythrus*
AC	1UGF5101016	Issa Valley	2016	*Papio cynocephalus*
AC	2UGF8101016	Issa Valley	2016	*Papio cynocephalus*
AD	2UGF8101016	Issa Valley	2016	*Papio cynocephalus*

Table S5. GenBank accession numbers for the sequences included into this study. (Excel Sheet)

Table S5. GenBank accession numbers of the sequence data generated in this study. Reference genotypes are shown in bold.

Seq Identifier	SAMPLE TYPE	TP0619	TP0548	TP0488	SPECIES	REGION
1SNM5120815	Swab	NA	**MK346879**	NA	Papio anubis	TZ
1UGF5101016	Skin	MK176936	**MK346886**	**MK346895**	Papio cynocephalus	TZ
2F8220307	Skin	NA	**MK346874**	NA	Papio anubis	TZ
2SNF2130815	Skin	MK176936	**MK346875**	**MK346901**	Papio anubis	TZ
2UGF8101016_1	Skin	MK176936	MK346886	**MK346900**	Papio cynocephalus	TZ
2UGF8101016_2	Skin	NA	NA	**MK346898**	Papio cynocephalus	TZ
3GNF8200916	Skin	NA	**MK346890**	**MK346917**	Papio anubis	TZ
3LMF5300415	Skin	**MK176936**	MK346874	NA	Papio anubis	TZ
3M8220307	Skin	MK176936	NA	**MK346909**	Papio anubis	TZ
4KNF2121016	Skin	MK176936	NA	**MK346899**	Chlorocebus pygerythrus	TZ
4LMF8160815	Skin	MK176936	MK346874	MK346909	Papio anubis	TZ
5RUM2080716	Skin	MK176936	**MK346889**	**MK346907**	Chlorocebus pygerythrus	TZ
5TNF11241215	Skin (mouth)	NA	**MK346878**	**MK346919**	Papio anubis	TZ
6LMF5170815	Skin	MK176936	MK346874	MK346909	Papio anubis	TZ
6RUM2090716	Skin	MK176936	**MK346888**	**MK346908**	Chlorocebus pygerythrus	TZ
6SNF2081115	Skin	MK176936	MK346875	MK346901	Papio anubis	TZ
7F5250307	Skin	MK176936	MK346874	MK346909	Papio anubis	TZ
7MNM1200916	Skin	MK176936	**MK346893**	NA	Chlorocebus pygerythrus	TZ
7SNM5081115	Skin	MK176936	**MK346880**	**MK346905**	Papio anubis	TZ
8UNM2010716	Skin	MK176936	**MK346884**	NA	Chlorocebus pygerythrus	TZ
9LMM2180815	Skin	MK176936	**MK346876**	MK346907	Cercopithecus mitis	TZ
9NCF8170516	Skin	MK176936	**MK346877**	NA	Papio anubis	TZ
10LMF8190815	Swab	NA	MK346874	NA	Papio anubis	TZ
10M2260307	Skin	NA	NA	MK346909	Papio anubis	TZ
10NCM8170516	Skin	MK176936	MK346877	MK346895	Papio anubis	TZ

11LMF5200815_1	Skin	MK176936	MK346874	**MK346914**	Papio anubis	TZ
11LMF5200815_2	Lymph node	NA	**MK346892**	NA	Papio anubis	TZ
12LMF2210815	Skin	MK176936	MK346874	**MK346902**	Papio anubis	TZ
12NCF5210317	Skin	MK176936	MK346877	MK346895	Papio anubis	TZ
12TNM9251215	Skin	MK176936	MK346878	NA	Papio anubis	TZ
13LMM8210815	Skin	MK176936	MK346874	MK346914	Papio anubis	TZ
13NCM2210317	Skin	MK176936	MK346877	NA	Papio anubis	TZ
14NCM5210317	Skin	MK176936	MK346877	MK346895	Papio anubis	TZ
14RUF5130716	Skin	MK176936	**MK346883**	MK346907	Papio cynocephalus	TZ
15F8270307	Skin	MK176936	MK346874	**MK346910**	Papio anubis	TZ
15NCF2220317	Skin	MK176936	MK346877	NA	Papio anubis	TZ
16LMM5250815	Skin	NA	MK346874	NA	Papio anubis	TZ
16M8280307	Skin	MK176936	MK346874	MK346909	Papio anubis	TZ
16NCF2220317	Skin	MK176936	MK346877	NA	Papio anubis	TZ
16RUF8140716	Skin	MK176936	MK346883	MK346907	Papio cynocephalus	TZ
17LMF5270815	Skin	MK176936	MK346874	NA	Papio anubis	TZ
17NCF2220317	Skin	MK176936	MK346877	**MK346897**	Papio anubis	TZ
17RUM5140716	Skin	NA	MK346883	NA	Papio cynocephalus	TZ
18LMF5270815	Skin	MK176936	MK346874	NA	Papio anubis	TZ
18NCF8220317	Skin	MK176936	MK346877	**MK346904**	Papio anubis	TZ
18RUF2140716	Skin	NA	NA	MK346907	Papio cynocephalus	TZ
19GNM2220916	Skin	NA	MK346890	MK346917	Papio anubis	TZ
19LMF8280815	Skin	MK176936	MK346874	MK346902	Papio anubis	TZ
20GNF5220916	Skin	MK176936	MK346890	**MK346918**	Papio anubis	TZ
20M5030407	Skin	MK176936	MK346874	NA	Papio anubis	TZ
21F8040407	Skin	MK176936	MK346874	MK346909	Papio anubis	TZ
21LMF2290815	Skin	MK176936	MK346874	**MK346911**	Papio anubis	TZ
22LMF5290815	Skin	MK176936	MK346874	MK346909	Papio anubis	TZ

24LMM2081215	Skin	MK176936	MK346874	MK346895	Papio anubis	TZ
24SNM5151115_1	Skin	MK176936	MK346879	**MK346896**	Papio anubis	TZ
24SNM5151115_2	Skin	NA	NA	**MK346912**	Papio anubis	TZ
26F8060407	Skin	MK176936	MK346874	**MK346915**	Papio anubis	TZ
27MKM5180715	Skin	NA	**MK346885**	NA	Papio cynocephalus	TZ
29SNF2191115	Skin	NA	MK346879	**MK346906**	Papio anubis	TZ
30LMF5190416	Skin	MK176936	MK346874	MK346902	Papio anubis	TZ
32F2110407	Skin	MK176936	MK346874	MK346909	Papio anubis	TZ
32LMM2190317	Skin	MK176936	**MK346887**	MK346904	Chlorocebus pygerythrus	TZ
33M8120407	Skin	MK176936	MK346874	MK346909	Papio anubis	TZ
34F2130407	Skin	MK176936	MK346874	**MK346913**	Papio anubis	TZ
34LMM2190317	Skin	MK176936	**MK346894**	MK346904	Chlorocebus pygerythrus	TZ
39M8150407	Skin	MK176936	MK346874	NA	Papio anubis	TZ
40M5160407	Skin	MK176936	MK346874	**LMNP-1 (CP021113.1:523,371 -524,125)**	Papio anubis	TZ
41SNM1231115	Skin	MK176936	**MK346891**	MK346895	Chlorocebus pygerythrus	TZ
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56F8230407	Skin	MK176936	MK346874	NA	Papio anubis	TZ
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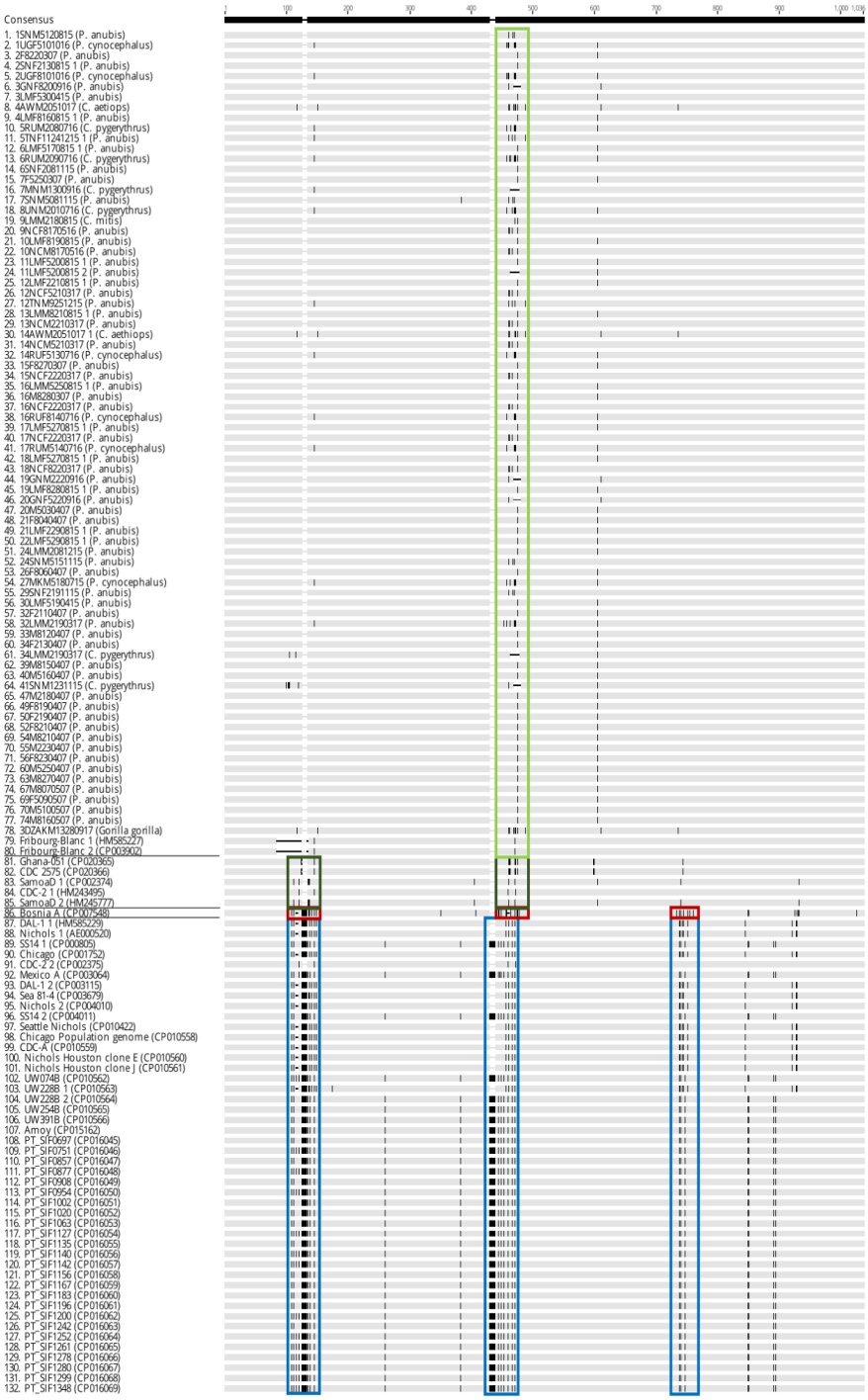
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5. 2UGF8101016 (P. cynocephalus)
6. 3GNF8200916 (P. anubis)
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8. 4AWM2051017 (C. aethiops)
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10. 5RUM2080716 (C. pygerythrus)
11. 5TNF1124215 1 (P. anubis)
12. 6LMF5170815 1 (P. anubis)
13. 6RUM2090716 (C. pygerythrus)
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29. 13NCM2210317 (P. anubis)
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80. Fribourg-Blanc 2 (CP003902)
81. Ghana-051 (CP020365)
82. CDC 2575 (CP020366)
83. SamoaD 1 (CP002374)
84. CDC-2 1 (HM243495)
85. SamoaD 2 (HM245777)
86. Bissau A (CP007528)
87. DAL-1 1 (HM585229)
88. Nichols 1 (AE000520)
89. SS14 1 (CP000805)
90. Chicago (CP001752)
91. CDC-2 2 (CP002375)
92. Mexico A (CP003064)
93. DAL-1 2 (CP003115)
94. Sea 81-4 (CP003679)
95. Nichols 2 (CP004010)
96. SS14 2 (CP004011)
97. Seattle Nichols (CP010422)
98. Chicago Population genome (CP010558)
99. CDC-A (CP010559)
100. Nichols Houston clone E (CP010560)
101. Nichols Houston clone J (CP010561)
102. UW074B (CP010562)
103. UW2288 1 (CP010563)
104. UW2288 2 (CP010564)
105. UW254B (CP010565)
106. UW391B (CP010566)
107. Amoy (CP015162)
108. PT_SIF0697 (CP016045)
109. PT_SIF0751 (CP016046)
110. PT_SIF0857 (CP016047)
111. PT_SIF0877 (CP016048)
112. PT_SIF0908 (CP016049)
113. PT_SIF0954 (CP016050)
114. PT_SIF1002 (CP016051)
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116. PT_SIF1063 (CP016053)
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121. PT_SIF1156 (CP016058)
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123. PT_SIF1183 (CP016060)
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125. PT_SIF1200 (CP016062)
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127. PT_SIF1252 (CP016064)
128. PT_SIF1261 (CP016065)
129. PT_SIF1278 (CP016066)
130. PT_SIF1280 (CP016067)
131. PT_SIF1299 (CP016068)
132. PT_SIF1348 (CP016069)

Figure S1. Alignment of NHP and human *TPA*, *TEN*, and *TPE* sequence data of *TP0548* locus. Sequences of *TP* of NHP origin show only one part of the sequence where most of the nucleotide variation is found (light green box). This distinguishes them from human *TPA* (blue box) and *TEN* (red box) as well as human yaws-causing strains (*TPE*; dark green box), where we have three and two variable regions, respectively. GenBank accession numbers for all NHP samples are listed in Table S5.

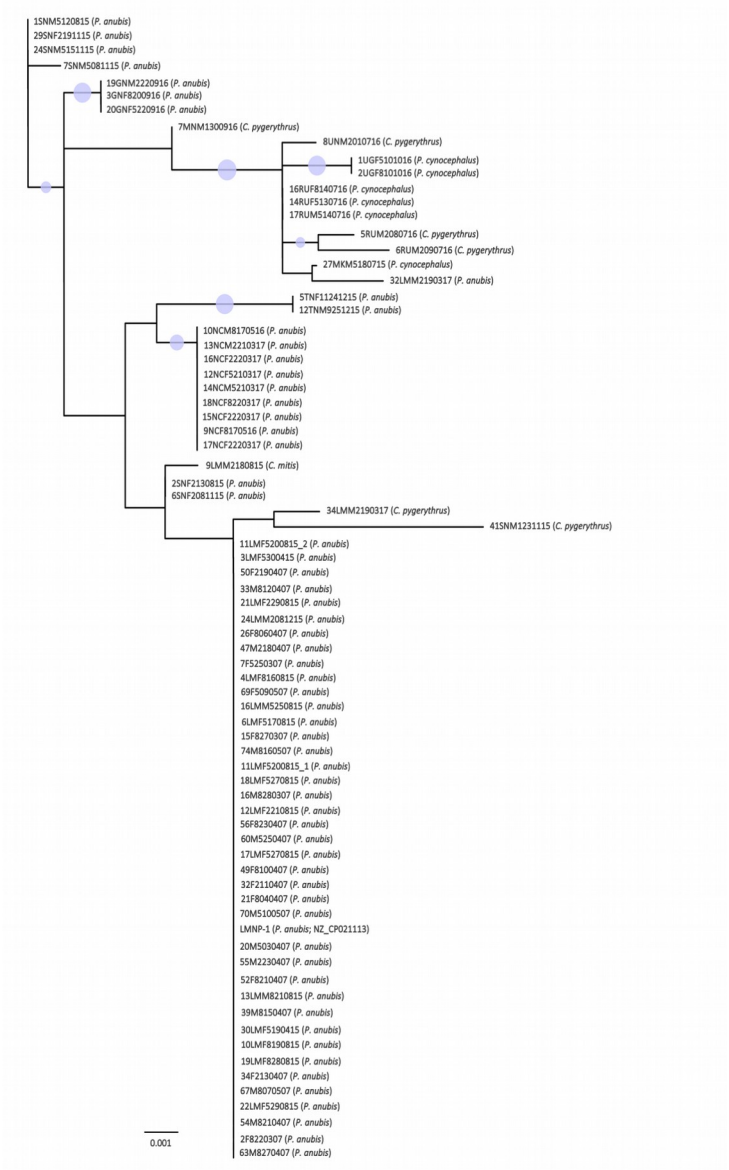
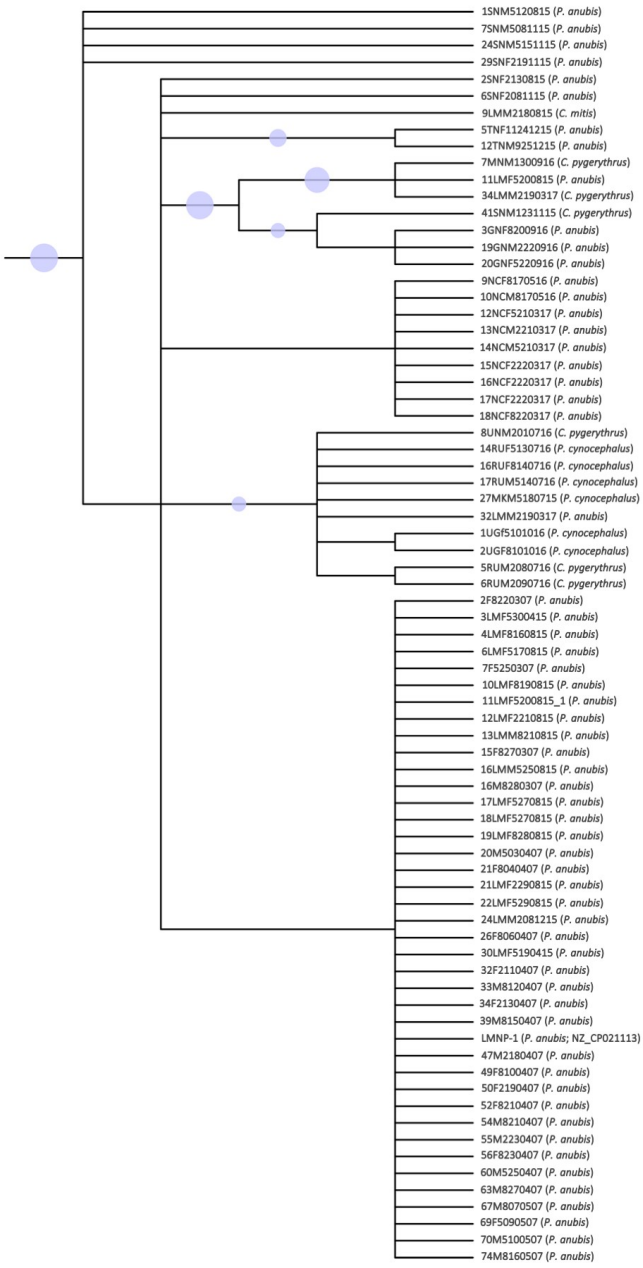


Figure S2. ML tree for the *TP0548* locus of the Tanzanian strains of NHP origin. 1,000 bootstrap replicates were performed and bootstrap values from 80-100% are highlighted as light blue circles of respective size. The bar indicates substitutions per site.



106

107 **Figure S3. MP tree for the *TP0548* locus of the Tanzanian strains of NHP origin.** 1,000

108 bootstrap replicates were performed and bootstrap values from 80-100% are highlighted as

109 light blue circles of respective size. Gaps were coded as fifth character.

Figure S4. ML tree for the *TP0488* locus of the Tanzanian strains of NHP origin. 1,000 bootstrap replicates were performed and bootstrap values from 80-100% are highlighted as light blue circles of respective size. The bar indicates substitutions per site.

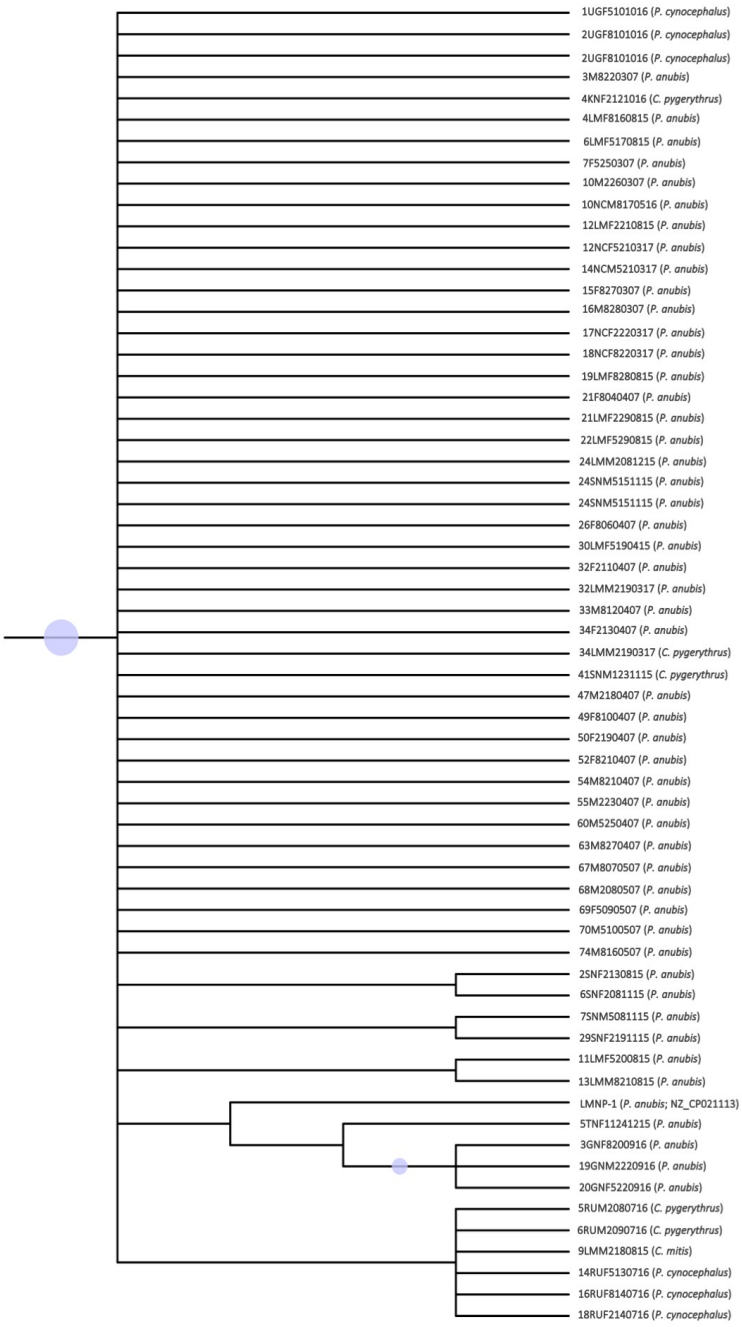


Figure S5. MP tree for the *TP0488* locus of the Tanzanian strains of NHP origin. 1,000 bootstrap replicates were performed and bootstrap values from 80-100% are highlighted as light blue circles of respective size. Gaps were coded as fifth character.

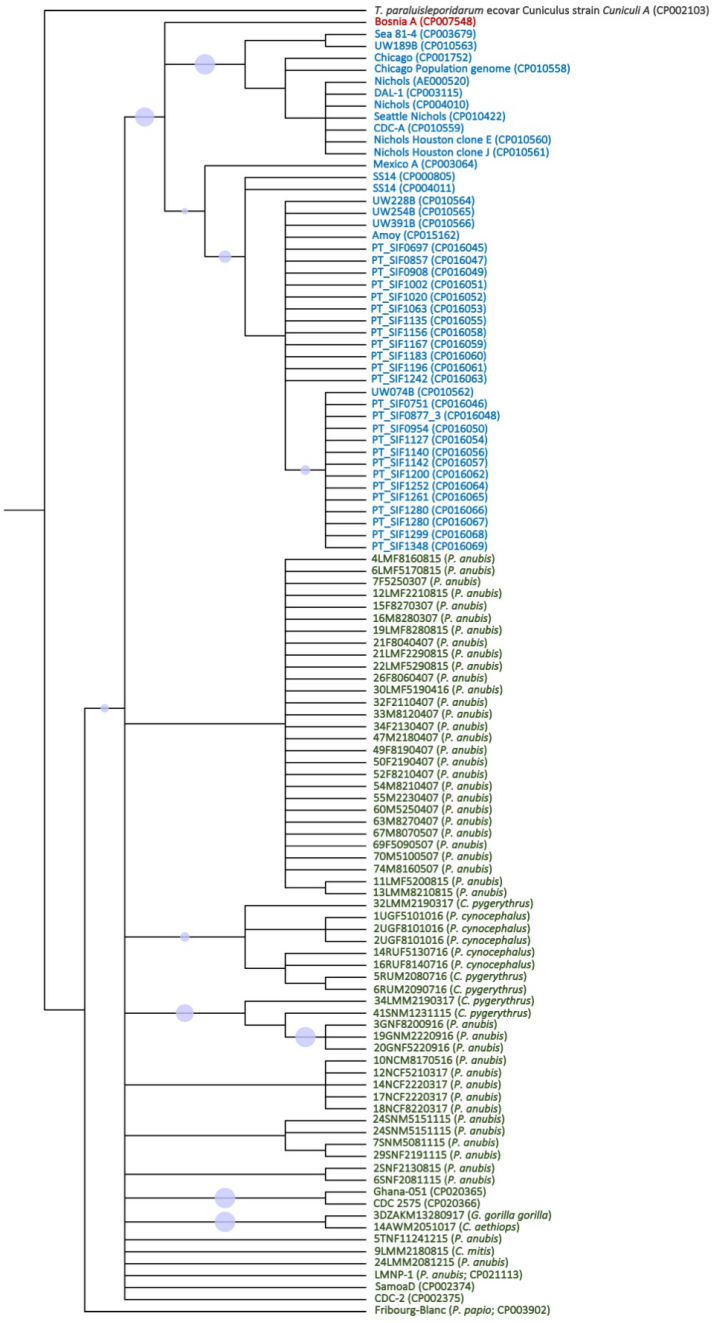


Figure S6. Rooted MP tree based on the concatenated sequences used for MLST (*TP0548* and *TP0488*). The tree is based on 1,773 nts and 1,000 bootstrap replicates.

Bootstrap values from 80-100% are highlighted as light blue circles of respective size. NHP species and/or GenBank accession numbers of published strains are provided in parentheses following the name of the strain. In all cases were the species is not mentioned, sequences are from *TP* of human origin. Blue=subsp. *pallidum*, green=subsp. *pertenue*, red=subsp. *endemicum*. The pathogen causing rabbit syphilis, *Treponema paraluisleporidarum* ecovar *Cuniculus* strain Cuniculi A, is used as an outgroup. The bar refers to substitutions per site. Gaps were coded as fifth character.

Figure S7. Camera trap photos of Western lowland gorillas with severe ulcerative skin lesions at Odzala-Kokoua National Park, Republic of the Congo, in 2017. Fecal samples analyzed in this study were collected in the same area and originated from animals with severe facial lesions. The images are not covered by the CC BY license. Image credits to African Parks (www.african-parks.org). All rights reserved, used with permission. When using the images online and across all platforms, African Parks and the photographer must be named appropriately, and African Parks and Odzala--Kokoua National Park should be mentioned using the following social media handles: on Twitter: @AfricanParks, Instagram: @AfricanParksNetwork and Facebook: @AfricanParks.Odzala--Kokoua National Park.

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CHAPTER FIVE

5.0 WILD NON-HUMAN PRIMATES IN TANZANIA HAVE ANTIBODIES REACTIVE WITH A WIDE RANGE OF VIRUS ANTIGENS

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Abstract

Viral infections are of major economic and public health concern as currently demonstrated by the Ebola epidemic in Central- and East-Africa. Our knowledge on diseases that are shared between humans and our closest relatives, the non-human primates (NHP), is still limited but is of great importance for the identification of disease reservoirs. Previous studies in bats showed that antibody screening approaches with full virus antigens are useful to detect past infections with already known and closely related viruses. In an explorative screening approach, we tested for antibody reactivity against viral antigens representing a number of selected and medically important human pathogenic viruses. A total of 74 NHPs (*Papio anubis* [n=48], *Papio cynocephalus* [n=10], *Chlorocebus pygerythrus* [n=13] and *Cercopithecus mitis* [n=2]) were chemically immobilized. Using remotely projected mixture of 10 mg ketamine hydrochloride /kg body mass and 0.05 mg Medetomidine hydrochloride /kg body mass in 3ml darts. The NHPs were sampled for whole blood in five different National Parks in Northern Tanzania. After centrifugation at 55,000 relative centrifugation force for 15minutes, the resultant serum samples were frozen tested for antibodies reactive against 20 full virus antigens or recombinant antigens using the MOSAIC indirect immunofluorescence test (IIFT)-Chip technology (Euroimmun). The viruses represented twelve virus families of public health relevance that can potentially be transmitted between humans and NHPs. Measles and Canine distemper viruses were rechecked by ELISA, virus neutralization and MelJuso cell line tests. Majority of the NHPs showed antibodies reactive against antigens from measles virus (89.2%, n=66), mouse hepatitis virus (78.4%, n= 58), mouse rotavirus (73.0%, n= 54), H1N1 Singapore (48.6%, n=36), yellow fever virus (37.8%, n=28), dengue virus (23.0%, n=17), adenovirus type 3 (21.6%, n= 16) and parainfluenza 2 virus (10.8%, n=8). None of the animals reacted with Ebola (EBOV), Hantavirus (DBOV), Crimean-Congo haemorrhagic fever (CCHF-GPC), Rift Valley Fever (RVF), La Crosse

(LACV), Sendai (SeV) and Sindbis (SINV) antigens. Amazingly, highly specific neutralization tests for measles virus were negative in all cases suggesting that NHP had encountered related paramyxoviruses in the past. Our exploratory serosurvey encourages further studies to detect, isolate and characterize the actual viruses responsible for the cross-reactive antibodies detected in Tanzanian NHPs.

Key words: baboons, monkeys, Tanzania, virus, zoonoses

5.1 Introduction

The world has recently witnessed increasing rates of disease transmission between humans and wildlife, contributing to threaten both public health and biodiversity conservation (Daszak *et al.,* 2000; Jones *et al.,* 2008). Among the main driving forces is human occupation of the new environments and disruption of ecosystems, especially the diverse tropical forests that remained unchanged for thousands of years and harbor potentially threatening microbes (Locatelli & Peeters, 2012). Non-human primates (NHPs) occupying these ecosystems are reservoirs for a wide range of both macro- and micro-parasites for humans, including a variety of helminths, protozoa, bacteria and viruses (Locatelli & Peeters, 2012).

NHPs are potentially important reservoirs and intermediate hosts in the transmission of pathogens from the wildlife in their natural settings to humans in the interfaces and beyond. For example, NHPs are involved in transmission of arthropod borne viruses (arboviruses) such as dengue fever, yellow fever, Zika, chikungunya and West Nile viruses (Weaver, 2013). However, little is known about other pathogens, including viruses rather than arboviruses, and the actual role the wild primates play in their transmission within and outside their natural ecosystems and in captivity. Due to their phylogenetic closeness to humans, NHPs share a number of pathogens with humans and more likely do play a

significant role in future emerging or re-emerging global pandemics. This may inflict massive economic losses in terms of ill health, death, treatment costs and loses attributable to inability of affected people to work. Several facilitating factors exist such as climate change, genetic changes (mutations and recombination), immunosuppression, emergence of antibiotic resistant pathogens in human and animal populations and increase in human travels. Chances of pathogens jumping from their original host species, invade and rapidly spreading to new species or new geographical areas are currently higher than before.

Nearly 75% of emerging infectious diseases of humans are zoonotic and majority originate from wildlife (Woolhouse and Gowtage-Sequeria, 2005; Cleveland et al., 2007). Over half of shared emerging pathogens in humans are viruses originating from wild NHPs (Jones *et al.*, 2008). Among well-known deadly viral pathogens of NHP origin include the Ebola virus, which cumulatively from 1976 to 2018, has affected 31,033 people and killed 12,913 including suspects, probable and confirmed cases (WHO, 2019). A number of studies have detected and tracked down various viral pathogens or diseases they cause. However, effective and sustainable control and eradication of viral diseases transmissible between humans, livestock and wild animals, unavoidably needs the use of a holistic and integrative One Health approach to identify drivers for human, animal and environmental health.

Studying virus diversity in animal populations might help predict future pandemics but is greatly challenged by the fact that most viruses cause short-lived viraemia. Therefore, nucleic acid detection assays and state-of-the art next generation sequencing techniques often fail to detect new viruses. To the contrary, antibody detection provides indirect evidence of past viral encounters even years after infection. In previous studies, serologic assays helped to address the viral diversity in bats and led to the discovery of ancestral

paramyxoviruses like Mumps and Measles (Drexler et al., 2012, Müller et al., 2016). Exploring the antibody landscape in animal populations is highly beneficial for targeted nucleic-acid detection-based studies.

This study was undertaken to determine viral pathogens of human health relevance in the wild NHPs in Tanzania to inform strategies for more effective prevention and control of potential zoonotic viral pathogens, diseases they cause and improve public health. We hypothesized that free-ranging Tanzanian NHPs are exposed to a wide range of viruses of public health significance and, as a response; they have developed antibodies against specific viral antigens. We aimed at detecting antibodies reactive against a total of twenty (20) different human viral antigens comprising 12 virus families, whose key characteristics are summarized in Appendix 1. Ideally, every targeted virus in this study represented all other members of its family and detection of antibodies against such a virus signifies NHP's exposure to or infection by the specific virus or any other member of its family.

The representative viruses were: Human Adenovirus (HAdV), Hantavirus (DBOV), Crimean-Congo haemorrhagic fever virus (CCHFV-GPC), Rift valley fever virus (RVFV), La Crosse virus (LACV), Cocksakievirus A24 (CVA24), Dengue virus (DENV), Yellow fever virus (YFV), Tick-borne encephalitis virus (TBEV), Human coronavirus strain 229E (HCoV 229E), Mouse hepatitis virus type 1 (MVH-1), Cowpox virus (CPXV), Ebola virus (ZEBOV), Minute virus of mice (MVM), Sendai virus (SeV), Picornavirus type 2 (PIV 2), Measles virus (MeV), Parainfluenza virus (H1N1 Singapore) and Sindbis virus (SINV). Very few studies were done on viruses of free-ranging NHPs in Tanzania. This study broadens our understanding of potential viral pathogens and diseases shared between humans and NHPs in Tanzania.

5.2 Material and Methods

5.2.1 Ethical statement

Relevant legal and institutional frameworks were observed in undertaking this research including adherence to the Veterinary Act No. 16 of 2003 and Tanzania Wildlife Research Institute's (TAWIRI) Guidelines for Conducting Wildlife Research (2012; http://tawiri.or.tz/wp-content/uploads/2017/05/Wildlife-research-guideline.pdf). The required permits were obtained from relevant authorities in Tanzania including: the Commission for Science and Technology in Tanzania (2015–89-NA-2014–228), Ministry for Natural Resources and Tourism (Wildlife Division, HA.403/563/01B/90, 178/606/01/115 and HA.178/606/01/6) and Tanzania National Parks (TNP/HQ/C.10/13). All the techniques and methods for animal handling and their associated protocols were reviewed and approved by the Animal Welfare and Ethics Committee of the German Primate Center (E10–17) and Sokoine University of Agriculture (SUA/ADM/R.1/8). The NHPs were handled by registered veterinarians and field team stayed with and closely monitored every anesthetized animal until full recovery, when the animal re-joined its group or at least when to defend itself against enemies.

5.2.2 Animals and sampling

Details on how the NHPs were captured and sample collection are found in Supplementary material 1.

5.2.3 Indirect Immunofluorescence Test (IIFT)

Indirect Immunofluorescence Test (MOSAIC IIFT-Chip technology, EUROIMMUN AG, Lübeck, Germany) was used to detect antibodies against 20 different viral antigens similar to previous bat-related studies (Muller et al., 2007; Drexler et al., 2012; Müller et al.

2016). Per incubation field, 70 µl of 1:40 diluted serum (serum: buffer) were placed on the slides and incubated in humid chamber at 37°C for 2 hours. This was followed by 3 to 5 times wash in phosphate buffered saline mixed with 0.1% Tween (PBS-T). The reactions were detected by addition of 70 µl of goat-anti-monkey immunoglobulin (Ig) diluted at 1:500 in sample buffer. The slides were then incubated at 37°C for 1 hour in humid chamber and then washed 3 to 5 times with PBS-T. A volume of 70 µl of donkey-anti-goat-CY3 1:200 diluted (final dilution; 1:100 from a 50% glycerol stock) in PBS and the slides incubated for 30 minutes at 37°C in humid chamber. Washing with PBS-T was done 3 to 5 times on a rocking table followed by one more wash with water before applying DAPI Gold mounting medium (Invitrogen). The slides were then stored in a cool and dark place until examination and analysis was performed with a Motic Immunofluorescence microscope (Zeiss, Jena, Germany). Three additional tests were performed to investigate the reactivity against Measles virus as briefly explained hereunder:

5.2.4 Enzyme Linked Immunosorbent Assay (ELISA)

To test the NHP sera for presence of Measles virus (MV)- specific IgG, ELISA was performed. The ELISA plates were coated with BPL-inactivated measles vaccine, incubated with a serially diluted serum, and developed with an anti-human IgG HRP-conjugated antibody. Although some background was visible at the lowest dilution (1:30 in this assay), the sera were all comparable to the two negative control monkey sera that were available in-house (shown in green). Serum from a MV-vaccinated macaque clearly tested positive (shown in red), confirming the validity of the assay.

5.2.5 Virus neutralization test

The NHP sera were tested for functionality in endpoint virus neutralization (VN) assay against both MV and Canine Distemper virus (CDV). In this assay, serum dilutions were

incubated with a set amount of live virus (MV and CDV, ±75 $TCID_{50}$) for 1 hour, and subsequently overlaid on Vero monolayers. The Vero cells used stably express dogSLAM, a receptor that can be used by both MV and CDV and are therefore susceptible to infection with both viruses. The MV and CDV used in these assays express fluorescent reporter proteins, so the VN assay was screened for fluorescence at 3 days post overlay of the serum/virus dilutions on cells.

5.2.6 MelJuso cell based assay

In the second assay, MelJuso cells were used that express the MV-F or H glycoproteins. The MelJuso cells were incubated with a set serum dilution, subsequently stained with an anti-human IgG FITC-labelled antibody, and cells acquired via flow cytometry. A negative control cell-line, not expressing any measles glycoprotein was also included in this assay.

5.2.7 Statistics

GraphPad Prism version 7.0c (GraphPad Software, La Jolla, CA, USA) was used to analyze the data of the detected NHP viruses. Proportions of different variables were compared and their statistical significance tested by using 2 x 2 contingency tables and a 2-tailed Fisher exact test. The χ^2-test was used to test for significant difference of the outcomes for more than two variables using n × 2 contingency tables. The proportions were tested at 0.05 and 95% CI and $p<0.05$ showed a significant statistical difference between or among the variables.

5.3 Results

The overall results on distribution of the reactions or cross reactions of NHP sera against full viral antigens are summarized in Fig. 5.1 while Park-specific and species-specific are in Fig. 5.2 A and B, respectively. A total of 13 viruses were detected out of 20 viruses of

public health relevance tested for and 7 viruses could not be detected (Figu. 5.1). Eight viruses were detected at frequencies between 10.8 and 89.2% and 5 viruses were below 10%. Geographically, NHPs in Lake Manyara and Mikumi National Parks (55%, 13/20 each) had significantly higher virus detection frequencies ($p<0.05$) than their counterparts in Arusha (15%, 3/20). However, the virus detection frequencies in Lake Manyara and Mikumi were comparable ($p>0.05$) to simian virus detection frequencies in Serengeti and Tarangire (40%, 8/20) (Fig. 5.2A). With respect to NHP species, virus detection frequencies observed in olive baboons (60%, 12/20) and yellow baboons (55%, 11/20) were comparable ($P>0.05$); however, both of these virus detection frequencies were significantly higher ($p<0.05$) than those of blue monkeys (15%, 3/20) and vervet monkeys (40%, 8/20) (Fig. 5.2B). With exception of Tick Borne Encephalitis virus (TBEV), which was detected only in the olive baboons, all other viruses that were detected in the olive baboons were also detected in yellow baboons. The most exceptional was Human Coronavirus 229E (HCoV 229E), NHP antibodies against which were detected only in the vervet monkeys of Serengeti National Park (Fig. 5.2 A & B).

Fig. 5.2 A summarizes all the viruses of human health significance that were detected in free-ranging NHPs across all five National Parks involved in the current study. While Measles (MeV), Mouse hepatitis Viruse (MHV-1) and Mouse Rotavirus were detected in NHPs in all the parks, TBEV was only detected in NHPs of Lake Manyara and Cowpox (CPXV) only in NHPs of Mikumi and Tarangire. Interestingly, all the NHPs tested negative for seemingly rare viruses namely: Ebola (EBOV), Hantavirus (DBOV), Crimean-Congo Haemorrhagic fever (CCHF-GPC), Rift Valley Fever (RVF), La Crosse (LACV), Sendai (SeV) and Sindbis (SINV) viruses.

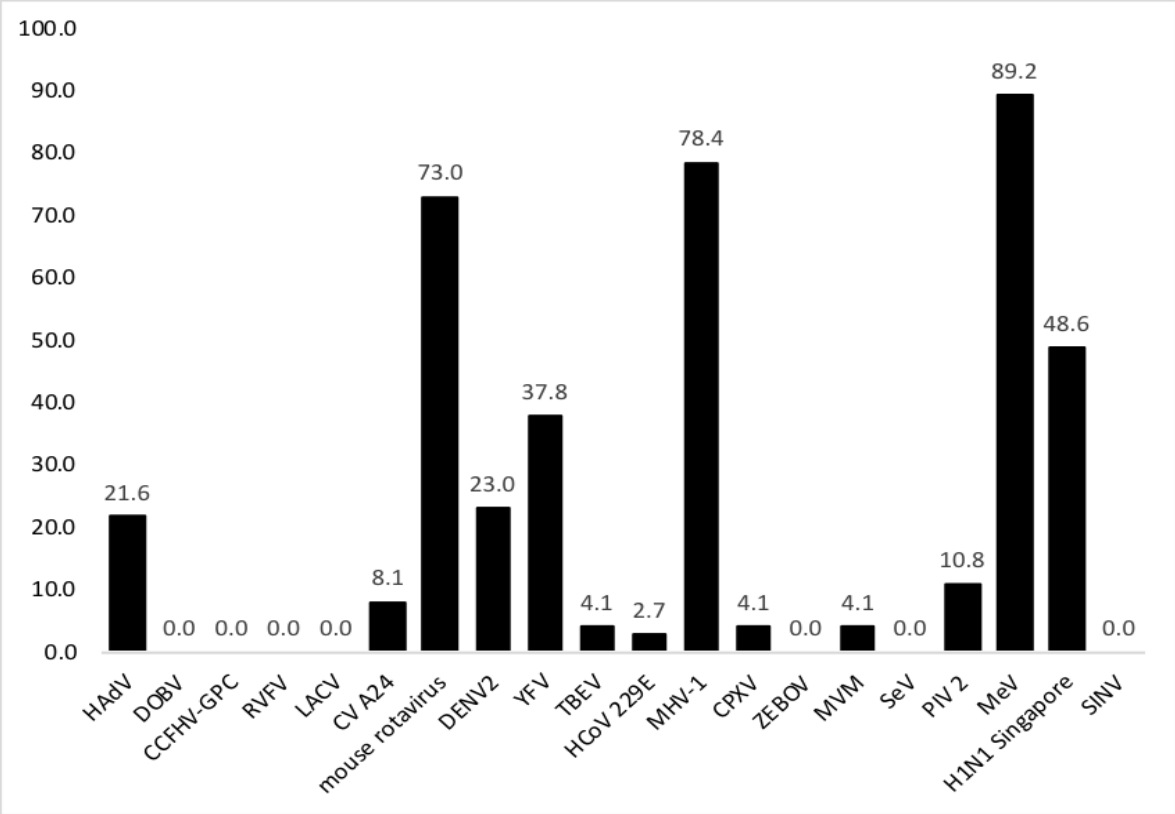
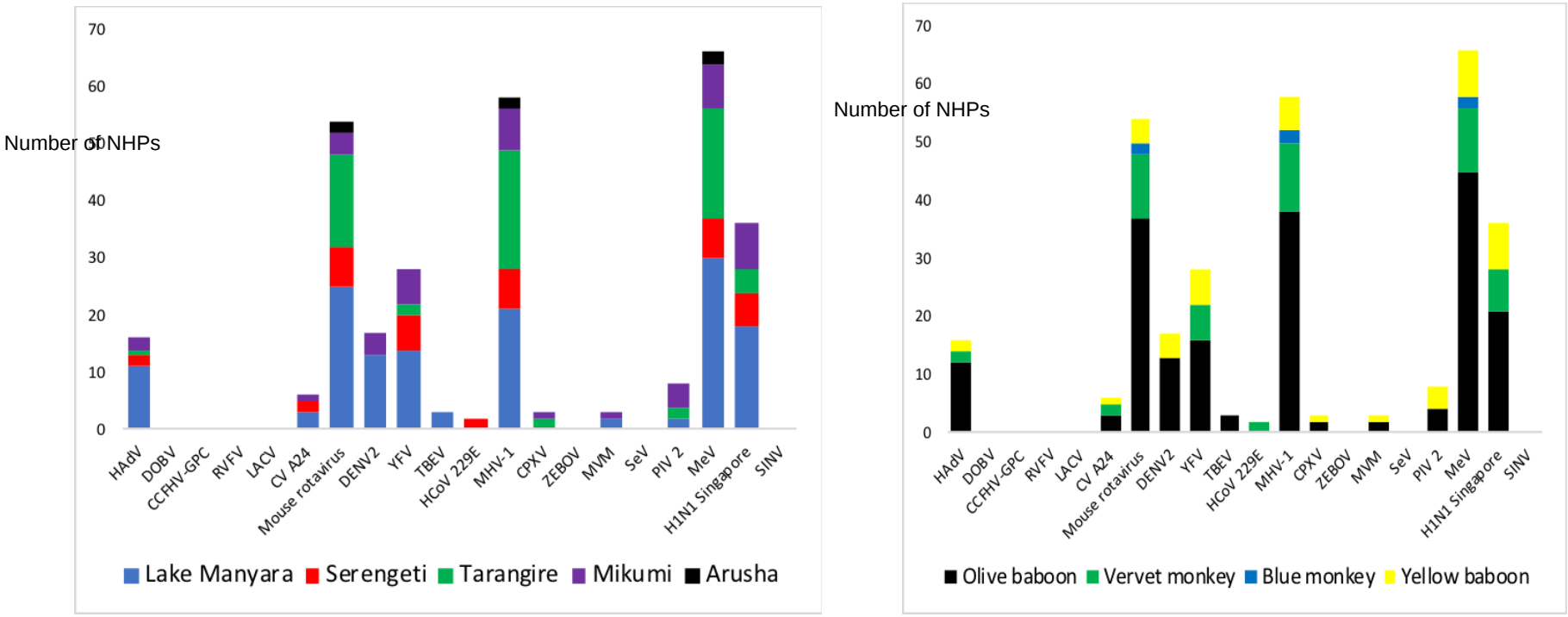


Figure 5.1 Frequency of antibody reactivity with 20 full virus antigens in free-ranging Tanzanian NHPs (%, n=74).

HAdV stands for Human Adenovirus, DBOV Hantavirus, CCHFV-GPC Crimean-Congo haemorrhagic fever virus, RVFV Rift valley fever virus, LACV La Crosse virus, CVA24 Cocksakievirus A24, DENV Dengue virus, YFV Yellow fever virus, TBEV Tick-borne encephalitis virus, HcoV 229E Human coronavirus type 229E, MVH-1 Mouse hepatitis virus type 1, CPXV Cowpox virus, ZEBOV Ebola virus, MVM Minute virus of mice, SeV Sendai virus, PIV 2 Picornavirus type 2, MeV Measles virus, H1N1 Singapore Parainfluenza virus H1N1 Singapore and SIV Sindbis virus.



A B

Figure 5.2 Origin and species of NHPs whose antibodies reacted or cross-reacted with full virus antigens (n=74)

The National Parks of origin (A) and species of NHPs (B); full names of the viruses were previously provided under Figure 5.1

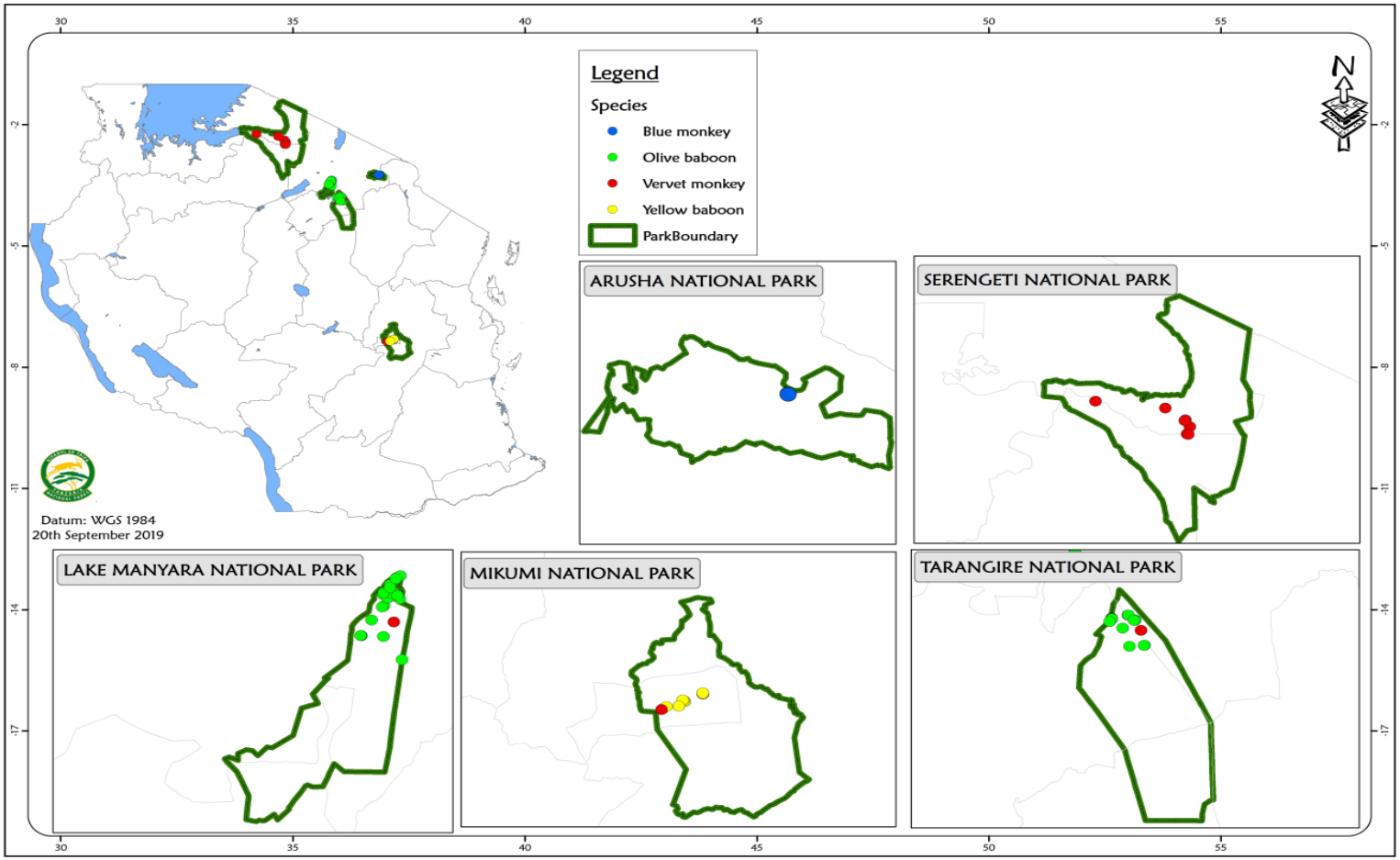
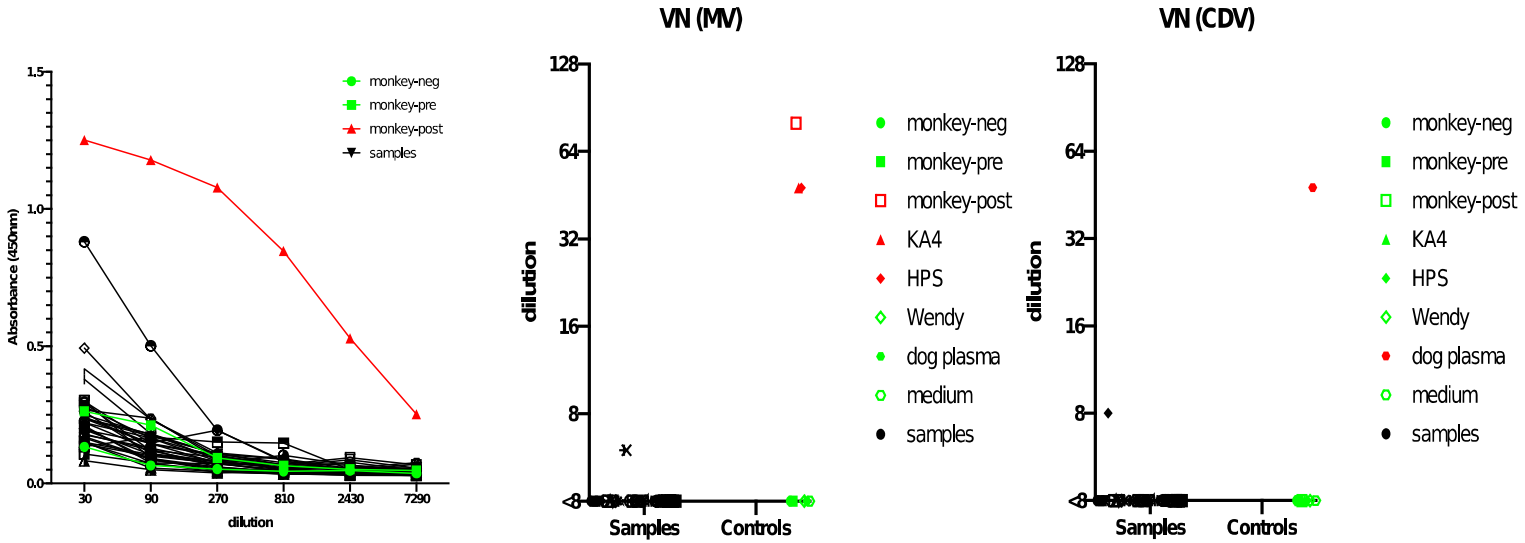

Legend
Species
Blue monkey
Olive baboon
Vervet monkey
Yellow baboon
ParkBoundary
N
Datum: WGS 1984
20th September 2019
ARUSHA NATIONAL PARK
SERENGETI NATIONAL PARK
LAKE MANYARA NATIONAL PARK
MIKUMI NATIONAL PARK
TARANGIRE NATIONAL PARK
30
35
40
45
50
55
-2
-5
-8
-11
-14
-17

Figure 5.3 Map of Tanzania showing NHP species and capture locations in the study National Parks



A B C

Figure 5.4 ELISA and virus neutralization test results

(A) ELISA test results: Although some background was visible at the lowest dilution (1:30 in this assay), all the tested NHP sera were comparable to the two negative control monkey sera that were available in-house (shown in green). Serum from a Measles virus (MV)-vaccinated macaque clearly tested positive (shown in red), confirming validity of the assay.

(B) Virus neutralization test for MV: All the tested NHP sera were negative for neutralizing activity against MV. In two samples (28LMM1081215 and 21TNF1311215), some neutralizing activity was detected. Again, all negative and positive controls scored as expected (shown in red and green), and back-titration of virus confirmed that the viral input was on point.

(C) Virus neutralization test for Canine Distemper Virus (CDV): All the tested NHP sera were negative for neutralizing activity against CDV. In one sample (22MKF1170715), some neutralizing activity was detected. Again, all negative and positive controls scored as expected (shown in red and green in the respective plots), and back-titration of virus confirmed that the viral input was on point.

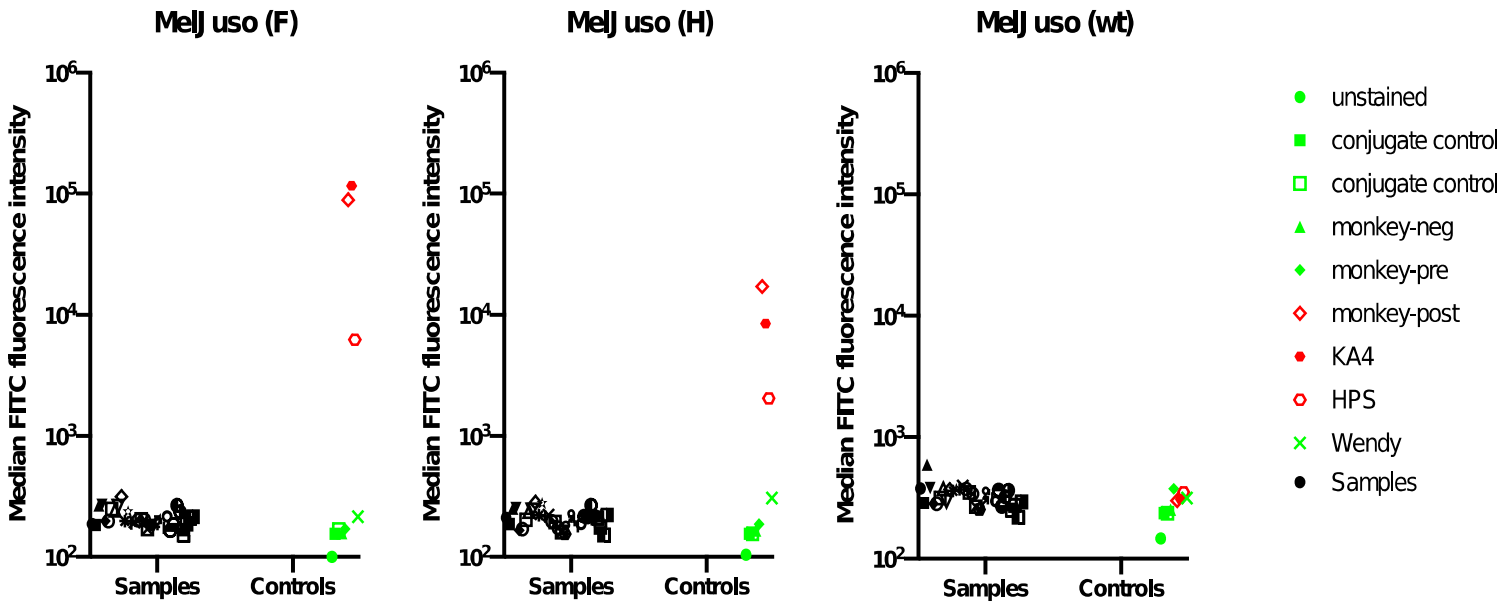


Figure 5.5 Results for MelJuso cell-line test for expression of F and H glycoproteins by Measles virus

All NHP sera tested negative for the presence of Measles virus F- and H-specific antibodies. Again, the positive controls (shown in red, both human and monkey MV-seropositive samples) tested clearly positive, whereas the negative controls (shown in green) tested clearly negative. On the wildtype MelJuso cell-line, not expressing measles glycoproteins, all samples tested negative as expected.

5.5 Discussion

Antibody detection provides indirect evidence of past viral encounters even years after the infection. Exploring the antibody landscape in animal populations is highly beneficial to make further studies that use more specific techniques such as nucleic-acid detection. In the current study, MOSAIC IIFT-Chip technology was used to detect antibodies in NHPs against full viral antigens from 20 different viruses of public health significance. This technique was successfully employed to detect viral antigens from bats in previous studies and key to discovery of the ancestral paramyxoviruses like Mumps and Measles (Muller et al., 2007; Drexler et al., 2012; Müller et al., 2016). However, among the shortfalls of this technique is failure to differentiate reactions between specific antibodies and cross reactions by other genetically or antigenically very closely related viruses. For example, the cross-reaction among members of Parvoviridae family that involved Minute Virus of Mice, Mouse parvovirus and other closely related parvoviruses (Janus *et al.*, 2008). Therefore, more specific tests such as nucleic acid detection, enzyme linked immunosorbent assay (ELISA), virus neutralization, culture and isolation using artificial media, cell lines and *in vivo* inoculation into animal models will help confirming the results and further identify the reacting or cross-reacting viruses.

In this study, the majority of NHPs (89.2 %) had antibodies reactive or cross-reactive with Measles viral antigens, which was not expected. In a similar serological study conducted in South Africa, Measles virus was also not detected in 27 wild baboons (Drewe et al., 2012). Further investigation by ELISA (Fig. 5.4 A), virus neutralization tests for Measles virus (Fig. 5.4 B) and Canine Distemper Virus (Fig. 5.4 C) and MelJuso cell line, which expresses Measles virus F and H glycoprotein (Fig. 5.5) were necessary to recheck these results. However, all these assays did not confirm presence of either Measles virus or Canine Distemper virus, a closely related virus.

Failure to confirm Measles virus and Canine distemper made us think of possible involvement of other Morbilliviruses - rinderpest and *Peste des Petits Ruminants* (PPR), that cause several important diseases of wild and domestic animals in Tanzania. While rinderpest is a deadly disease-causing mass die-offs of large ruminants, especially cattle, buffaloes and wildebeests; PPR affects domestic and wild small ruminants. Rinderpest has been eradicated and is currently not found in Tanzania as the country was declared free from the disease since 1998 (Ministry of Water and Livestock Development, 2002). The NHPs could have had been exposed to and build up immunity against rinderpest viruses previously when the disease was escalating in the country. If present, the rinderpest virus would have not gone unnoticedly without causing the disease in susceptible ruminant species. Therefore, there is a minimal chance for the NHPs to have recent exposure to the rinderpest virus.

The NHPs could have also been exposed to the PPR virus, which is circulating and causes economic losses in the country up to now. In theory, it can be speculated that Tanzanian NHPs could have been exposed to these wide-spread Morbilliviruses or other genetically/antigenically related viruses, react with and built up the antibodies detected in this study. Alternatively, probably there were non-specific reactions in the current study due to cross reactivity by other viruses related to Measles virus or interference from unknown test factors attributed to the positivity of the test.

Elsewhere in the world, the first cases of naturally occurring canine distemper virus (CDV) infections in NHPs were recorded in Japanese macaques (*Macaca fuscata*), rhesus monkeys (*Macaca mulatta*) and long-tailed macaques (*Macaca fascicularis*) in 1989, 2006 and 2008, respectively (Yoshikawa et al., 1989; Sun et al., 2010; Qiu et al., 2011; Sakai et al., 2013). In those studies, the affected NHPs had measles-like signs such as respiratory

distress, anorexia, fever, rash and conjunctivitis. In the current study, symptoms like these were not observed in any of the NHP populations sampled in Tanzania. Elsewhere, virus transmission by contact between laboratories that keep monkeys and wild monkeys or a spillover of CDV from a stray dog followed by adaptation to the new host were among the suspected sources of infection (Qiu et al., 2011). The CDV infection in primates raises potential risks for human infection especially people that lack cross-protective measles immunity (de Vries et al., 2014; Ludlow et al., 2014). Although further investigations to determine the causes of reactivity against MeV antigens were beyond the scope of the current study, investigations in these lines are warranted.

Mouse hepatitis virus (MHV) is an emerging infectious disease pathogen (Bergmann et al., 2006) with corona-like large peplomers or spike glycoproteins (S) used for attachment to the host cell (Snijder et al., 2003). Coronaviruses cause important respiratory and enteric diseases in human and animal populations by infecting epithelial cells of these systems and affect many animal species (Holmes, 2001). Coronaviruses of animal species such as porcine, feline, canine, murine and bovine infect intestinal tissue (Holmes, 2001). These viruses cause interstitial pneumonia with fever and sometimes diarrhea in humans (Nicholls et al., 2003; Peiris et al., 2003). Of the NHPs (all four species in the five sites), 78.4% were positive for antibodies against Mouse hepatitis virus type 1 (MHV-1) while 2.7% (two vervet monkeys from Serengeti, 34SNF1211115 and 40SNM1231115) had antibodies reactive or cross-reactive with antigens from human coronavirus (HCoV 229E). These NHPs in Tanzania were exposed to and developed immunity against coronaviruses (MHV-1 and HCoV 229E) or other closely related viruses antigenically. The animals that tested positive for HCoV 229E came from around the residential areas with frequent interactions with humans. However, the NHPs that tested positive for MHV-1 came from

populations that live in close proximity to and interact with humans while others were from locations of the Parks without direct interactions with humans.

Rotaviruses infect epithelial cells of mature villi in the small intestine leading to fever, nausea, malaise, headache, abdominal cramping, vomiting and diarrhea following spread by faecal-oral route (Otsyula et al., 1996; Anderson and Weber, 2004). In the current study, majority of NHPs (74.3%) had antibodies reactive or cross-reactive with mouse rotavirus antigen. NHPs do occasionally hunt and eat meat of various wild and domestic animal species including other primate species, antelopes, bird species and rodents such as mouse. Faecal-oral contamination is also not uncommon in the wild environments; most likely this increases chances for spreading rotavirus infection among the NHPs, especially in the areas where NHP population density is higher.

We found 47.3% of the NHPs in the current study positive for antibodies against H1N1 Singapore and this was consistent across all the five Parks and involved all four NHP species. Similar findings were obtained by Bunuma and others (2018), who detected H1N1 and H3N2 in olive baboons at different locations in Kenya at comparatively lower frequencies between 9 and 44%, except only Olorbototo, which had 75%. The Influenza infection was linked to close handling of the olive baboons by humans for 1 to 2 years; hence, the baboon infection had anthropozoonotic origin (Bunuma et al., 2018). In human-wildlife interfaces of Tanzania and elsewhere in Africa, NHPs are widely distributed, live in close proximity and frequently interact with humans, and often result into human-wildlife conflicts, bush meat hunting and preparations, feeding wild animals and contamination of utensil and residential environments by NHPs. Elsewhere in the world, Karlson and others (2013) detected H1N1 and H3N2 influenza A strains in performing macaques in Cambodia (29.2%), Singapore (16.7%), Sulawesi (16.1%), Bangladesh

(13.3%), and Java (6.0%). These performing macaques were closely interacting with people and offered almost ideal environments with higher possibility for disease transmission between the NHPs and humans.

Antibodies reactive or cross-reactive with Yellow Fever Virus (YFV) antigen were detected in 37.8% of the NHPs that making up three species namely yellow baboons in Mikumi as well as olive baboons and vervet monkeys of Lake Manyara and Serengeti. Being a re-emerging, zoonotic, noncontagious viral hemorrhagic disease endemic to Africa and South America, YFV infects human and non-human primates (Monath and Vasconcelos, 2015). *Haemagogus* and *Sabethes* mosquitoes are responsible for sylvatic cycle and *Aedes aegypti*, urban cycle of YF (Berret and Monath, 2003). The WHO receives reports of approximately 5,000 cases from Africa and 300 others from South America; however, estimation of the actual incidence rate stands at 10–50 fold higher (Monath, 2001). The natural transmission cycle of YF involves tree-hole breeding mosquitoes and a wide range of NHP species including monkeys, apes and marmosets (Monath, 2001). In Africa, NHPs rarely develop fatal disease following YFV infection (Jentes et al., 2010) while several New World monkey species in the Americas are susceptible and suffer from severe and fatal disease (Almeida *et al.*, 2014).

Dengue virus (DENV) causes a self-limiting and relatively mild febrile illness, dengue fever and dengue haemorrhagic fever transmitted to humans by *Aedes* mosquitoes (Gubler, 1998). DENV infects 50–100 million humans annually in tropical and subtropical regions, posing a considerable threat to public health in over 100 countries (Simmons et al., 2012). Dengue haemorrhagic fever (DHF) may lead to life-threatening dengue shock syndrome (DSS) and, if untreated, mortality rates exceed 20% though with proper medical care the rates decrease to less than 1% (WHO, 2017). Of all NHPs in this study, 24.3% had

antibodies reactive or cross reactive to DENV2. Although both human and NHPs are infected by Dengue viruses, sylvatic DENV which circulates in NHPs is distinguished from urban DENV in humans (Vasilakis et al, 2011; Rosi et al., 2012; Durbin et al., 2013). If frequent zoonotic transmission of dengue exists between NHPs and humans, the disease would have been more prevalent in or around human-wildlife interfaces, which is not the case in our study area. This finding therefore supports the hypothesis that separate sylvatic and urban cycles of DENV infection exists. This hypothesis is further affirmed by Mweya and others (2016), who model-predicted for high habitat suitability for the vectors *Aedes aegypti* and high risk of the infection was restricted along the coastal belt of Tanzania, specifically Dar es Salaam, Pwani, Morogoro, Tanga, Zanzibar, Lindi and Ruvuma regions.

Adenoviruses are frequent causes of fevers, upper respiratory tract symptoms, conjunctivitis, pneumonia, acute and chronic appendicitis, and bronchiolitis and are ubiquitous in environments contaminated by human faeces or sewage (WHO, 2004). They often cause mild and self-limiting infections though several fatal cases have been reported. Primate adenovirus from the New World monkeys have the potential to infect humans (Wevers et al., 2011; Kohl et al., 2012). In a study by Wevers and others (2011), one HAdVs positive sample came from a baboon of Lake Manyara, Tanzania. Detection of adenoviruses in the current study indicates that these adenoviruses are still circulating in baboons of Lake Manyara ever since. Adenoviruses from chimpanzees showed a remarkably close relationship to human adenoviruses, possibly indicating recent interspecies transmission (Wevers et al., 2011). High asymptomatic shedding of live adenovirus by NHPs intensifies environmental contamination and increases risks for infections and potential zoonotic transmission of these viruses.

The NHPs making up 20.3% composed of all four species in all the Parks were positive for antibodies against HAdVs. In the protected areas studied, probably HAdVs are widespread and easily transmitted within and across NHP species. By virtue of their being resistant to chemical or physical agents, adverse pH conditions, tertiary treatment and UV radiation of urban wastewater, adenoviruses survive outside the host body for long time (Thompson et al. 2003; Thurston-Enriquez et al. 2003). There are four genera under this family namely Mastadenovirus, Aviadenovirus, Atadenovirus and Siadenovirus (WHO, 2004) and over 60 HAdV types in seven species categorized by letters A–G with 51 antigenic types have been described based on their physical, chemical and biological properties (Buckwalter et al., 2012; Huang and Xu, 2013; WHO, 2004). In addition, based on the genomic sequencing and bioinformatic analysis, new HAdV types 52–68 have been discovered (Huang and Xu, 2013).

Most members identified in the first two decades of the AIDS epidemic belong to HAdV-D (De Jong et al., 1999). Almost 10 % of global pediatric respiratory illnesses are linked to species B, C and E whereas others are associated with gastrointestinal, urogenital and ocular infections (Sharma et al, 2009; Tebruegge and Curtis, 2012). Interestingly, Roy et al (2009) clearly classified ape adenoviruses into species corresponding to HAdV B, C and E. This finding shows that HAdVs may, most likely, be shared between humans and NHPs. Furthermore, intraspecies recombination between adenoviruses and phylogenetic relatedness across various primate hosts support existence of cross species transmission events in the history of B and E viruses (Roy et al., 2009).

The rest of the viruses including Parainfluenza (PIV 2), Coxakievirus (CVA24), Tick borne encephalitis (TBEV), Minute virus of mice (MVM 1), Cowpox (CPXV) and human coronavirus (HCoV 229E) were detected in frequencies below 10% of the NHPs showing

their rarity in free-ranging NHPs. These rare viruses have not been discussed in the current study.

Interestingly, however, all the NHPs tested negative for Ebola (EBOV), Hantavirus (DBOV), Crimean-Congo haemorrhagic fever (CCHF-GPC), Rift Valley Fever (RVF), La Crosse (LACV), Sendai (SeV) and Sindbis (SINV) virus antigens. Absence of these viruses in Tanzanian NHPs shows that these wild primates have not been adequately exposed to these viruses or their genetically or antigenically closely related ones. For example, absence of Ebola in NHPs is not surprising as it is in line with the absence of this disease in humans in Tanzania. Ebola does not only affect and threaten humans in Africa and elsewhere but also NHPs in the wild. For example, Ebola affected and decimated the endangered lowland gorillas (*Gorilla gorilla*) in Odzala-Kokoua National Park, DRC to extent of making the International Union for Conservation of Nature (IUCN) upgrade their conservation status from 'endangered' to 'critically endangered' in the 2007 Red List of Threatened Species (Hopkin, 2007). Generally, the great apes seem to be more prone to Ebola than smaller NHP species such as monkeys. Genetic and biological closeness of the great apes to humans may have been influencing their higher susceptibility to Ebola than the monkeys.

In conclusion, the current study has shown that Tanzanian NHPs were exposed to several viruses of public health relevance. *T. pallidum* infection and the detected viral infections occur independently in different NHP species and wildlife protected areas of Tanzania. People living and /working in or nearby all areas with close interactions between humans and NHPs need to take precautions to minimize chances for zoonotic transmission of these viruses and other pathogens. Among others, improving personal and environmental hygiene, control of vermin and disease vectors, proper use of PPEs when handling NHPs,

avoiding hunting for bushmeat and other wildlife products, provision of wildlife conservation and public health education will help minimizing the risks. Further researches are needed to establish whether or not the detected viruses are transmitted from NHPs to humans and/or vice versa, which was beyond the scope of the current study.

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APPENDICES

Appendix 1: Characteristics of the viruses whose full viral antigens reacted with antibodies from the NHPs

Virus name (abbreviation, Family)	Nucleic acid materials	Diseases caused (affected hosts)	Transmission mode	NHP infection	Closely related viruses	Reference
Measles virus, (MeV, Paramyxxoviridae)	Enveloped, single stranded RNA	Measles (humans),	Highly contagious by respiratory route from secretions	Yes (Macaques spp): Measles-like (respiratory distress, anorexia, fever, rash and conjunctivitis)	Canine distemper, rinderpest and Peste des petits ruminants (PPR)	Yoshikawa *et al*., 1989; Qiu *et al.*, 2011; Sun *et al.*, 2010; Sakai *et al.*, 2013; Laksono et al., 2016
Mouse Rotavirus	Non-enveloped, double-stranded RNA	Gastroenteritis, diarrhoea (humans, NHPs and)	Faecal-oral route	Yes	Other rotaviruses	Otsyula et al., 1996
Human Adenoviruses, (HAdV Adenoviridae)	Nonenveloped double-stranded DNA	Pediatric respiratory illnesses are (species B, C and E; others cause gastrointestinal, urogenital and ocular infections	Faecal-oral route	Fevers, upper respiratory tract symptoms, conjunctivitis, pneumonia, acute and chronic appendicitis, and bronchiolitis	Other types of HAdV (n-68)	Sharma et al, 2009; Tebruegge and Curtis, 2012
H1N1 Singapore (H1N1, Orthomyxoviridae)	Single stranded RNA	Influenza (humans)	Direct contact with secretions, aerosolized respiratory droplets and indirect contact with fomites.	Yes (Macaque spp), baboons	Other Influenza A virus types, Influenza C and D viruses	Bailey et al., 2018; Bunuma et al., 2018; WHO, 2019a;
Dengue virus type 2 (DENV, Flaviviridae)	Single stranded RNA	Female mosquitoes mainly *Aedes aegypti* and, to lesser extents, *Aedes albopictus*	Dengue (humans) including Dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS)	Yes (sylvatic form circulates in the NHPs)	DENV type 1, 3 and 4	Vasilakis et al, 2011; Durbin et, 2013; Rosi et al., 2012; WHO, 2019b

Tick borne encephalitis virus (TBEV, Flaviviridae)	Single stranded RNA	Infective bites by hard ticks (Ixodidae), rodents, mammals including dogs, cattle, sheep and goats and unpasteurized milk	Meningitis, encephalitis and meningoencephalitis (humans)	Not known	Omsk haemorrhagic fever virus, Kyasanur Forest disease virus, Alkhurma virus, looping ill virus and Powassan virus	Gritsun et al., 2003; CDC, 2014; Shi et al., 2018
Yellow Fever virus (YFV, Flaviviridae)	Enveloped, single stranded RNA	Infective bites by mosquitoes (Haemagogus, Sabethes and *Aedes aegypti*)	Yellow fever (humans)	Yes (monkeys, apes, marmosets, unspecified NHP spp.)	Other Flaviviruses (DENV, Zika, etc)	Weaver, 2013; Gardner and Ryman, 2010; Fernandes et al., 2017
Rift Valley Fever (RVF, Bunyaviridae)	Enveloped, single stranded RNA	Mosquito species (*Aedes*, *Anopheles*, *Culex*, *Eretmapoites*, *Mansonia*), other vectors e.g. sandflies and direct contacts with materials from infected animals	Rift valley fever. In humans, the symptoms range from uncomplicated acute febrile illness to retinitis, hepatitis, renal failure, meningoencephalitis, severe hemorrhagic disease, and death. In animals, necrotic hepatitis, hemorrhage and abortion, death of up to 100% among newborn animals	Yes (experimentally in macaques)	Other Bunyaviruses	Fontenille et al., 1998; Moutailler et al., 2008; Bird et al., 2009; Bouloy and Weber, 2010; Morrill and McClain, 1996; Wilson et al., 1994
Mouse hepatitis virus -1, MHV-1 (Coronaviridae)	Enveloped, single stranded RNA	Aerosol, direct contact, fomites and experimentally via transplantable tumors and placenta	Respiratory and enteric diseases with fever and diarrhea n humans and animals	Not known	Other Coronaviruses	Baker, 1998; Parker and Richter, 1978; Holmes 2008; Snijder et al., 2003; Taguchi and Hirai, 2012; Nicholls et al., 2003; Peiris et al., 2003

Sendai virus (SeV, Paramyxoviridae)	Single stranded RNA	Aerosal and direct contact with contaminated fomites	Non-pathogenic to humans; used as a model for other infectious pathogens	Yes	Other paramyxovirues	Durbin *et al*., 2000; Flecknell et al., 1983.
Hantavirus (DBOV, Bunyaviridae)	Sigle stranded RNA	Aerosol and direct contact with infected people (suggested), rodents or fomites	Pulmonary syndrome (humans), and haemorrhagic fever with renal syndrome	Yes (experimentally macaque monkeys and chimpanzees)	Other viruse under the family Bunyaviridae	Yanagihara et al., 1988
Crimean-Congo haemorrhagic fever (CCHFV, Bunyaviridae)	Sigle stranded RNA	Tick bites or contacts with infected people or animals	Haemorrhagic fever (humans) and unnoticedly circulates in various animal spp. including: giraffe, black rhinoceros, white rhinoceros, eland, buffalo, kudu, zebra, hares, rodents, wild carnivores and domestic dogs.	Yes, but are refractory or undergo mild infection with transient viraemia	Other Bunyaviruses	Shephard et al., 1987: Haddock et al., 2018
La Crosse virus (LACV, Bunyaviridae)		Mosquitoes (Aedes spp): they feed on Eastern chipmunks) and Eastern gray squirrels are amplifying hosts in US	Meningoencephalitis, fever, headache, vomiting and mental illness (humans, mainly children). Majority of infections are mild and scribed as forms are ‘flu’ or ‘summer cold’	Not known	Other Bunyaviruses	Gauld et al., 1975; Baldridge et al., 1989; Nasci et al., 2000; Calisher, 1994; Rust et al., 1999; Mc Junkin et al., 2001; Jones et al., 1999
Coxackievirus (CV A24, Picronaviridae	RNA	Direct contact (nasal and throat secretions, fecal-oral route and inhalation)	Acute haemorrhagic conjunctivitis, meningitis, myocarditis and pericarditis in humans	Not known	Other picornaviruses	Craighead, 2000; Mandell, 2000

Cowpox virus (CPXV, Poxviridae)	Double stranded DNA	Direct contact with infected animals especially rodents, which are reservoirs.	Smallpox (*Variola virus*), *Monkeypox Cowpox*	Yes (the virus was isolated from cheetah, jaguarundi, lion, panther, beaver, marmoset, mongoose, and rats.	Mouse parvovirus,	King et al., 2012; Mercer et al., 2007; Baxby et al., 1982; Marennikova et al., 1977; Gehring et al., 1972
Sindbis virus (SINV, Togaviridae)	Enveloped RNA	Ornithophilic mosquitoes (Culex spp) and contacts with the reservoirs birds (grouse and passerines) that are probable amplifying hosts while migratory birds may carry the virus over long distances	Rash-arthritis syndrome in humans; the clinical disease is known as Pogosta disease in Finland, Ockelbo disease in Sweden and Karelian fever in Russia.	Not known	Chikungunya virus	European Centre for Disease Control and Prevention, 2019.
Minute virus of mice (MVM, Parvoviridae)	Single stranded DNA	Contacts with laboratory and wild mice	In animals, hamsters and rats are susceptible to experimental infection	Not known	Mouse parvovirus and other closely related parvoviruses (cross reacts with MVM in Indirect immunofluorescent assay, IIFA)	Siegel, 1984; Janus et al., 2008; Kilham and Margolis, 1970, 1971; Nicklas et al., 1993; Crawford, 1966; Bonnard et al., 1976

Appendix 2: Supplementary material 1: Animals and sampling

For economic reasons, sera from 74 NHPs making up 25% of a pool of 289 NHPs previously sampled were randomly selected and analyzed in the current study. The NHPs included 49 olive baboons (*Papio anubis*), 13 vervet monkeys (*Chlorocebus pygerythrus*), 10 yellow baboons (*Papio cynocephalus*) and 2 blue monkeys (*Cercopithecus mitis*) from five National Parks, namely Serengeti (n-8), Arusha (n-2), Tarangire (n-21), Lake Manyara (n=32) and Mikumi (n=11).

Whole blood was collected from the femoral veins of the immobilized NHPs following a standardized protocol previously applied for baboons (Knauf *et al.*, 2011). Briefly, NHPs were chemically immobilized at a variable distance by using a remote projectile system (MOD JM, Dan-Inject ApS, Børkop, Denmark) targeting good muscles around shoulders and hindquarters. A dosage of 8.0-10.0 mg ketamine/kg body mass (Kyron Laboratories, Johannesburg, South Africa) in combination with 0.2 mg/kg medetomidine (Domitor; Pfizer, Berlin, Germany) was used and vital parameters including respirations, pulse frequency, and internal/ rectal body temperature were regularly measured and recorded roughly after every five minutes. Monitoring of pulse frequency and blood oxygen saturation was done using a Nellcor OxiMax N65 Pulse Oximeter (Tyco Healthcare Deutschland GmbH, Neustadt, Germany) and every anesthetized animal underwent a standardized health examination.

S-Monovette closed blood collection system (Sarstedt, Nümbrecht, Germany) mounted with a 20G needle was used whereby, after asepsis by swabbing the site with 70% ethanol, two 9-mL serum tubes that had no anticoagulants were filled with whole blood from a

total of 74 NHPs. The blood sample in the tubes was stored at 4°C and transported back to the base station at the end of the day for further processing. The whole blood in serum tubes was centrifuged at 55,000 relative centrifugation force for 15 min and the resultant sera from each NHP was separated and dispensed into a 2ml screw-capped cryovial. The cryovials were then stored in liquid nitrogen (MVE, USA) and then transferred into -80°C freezer at the Tanzania Wildlife Research Institute headquarters in Arusha, Tanzania. Furthermore, sets of the aliquots were exported to the laboratories for further analysis at DPZ in Göttingen and at the Institute of Virology in Berlin, Germany.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has shown that *T. pallidum* infection is geographically widespread across Tanzania and this bacterium, specifically TPE, infects NHPs of the Old World Monkey species, namely olive and yellow baboons, vervet monkeys and blue monkeys. The NHPs tested positive for TP infection in 11 of 14 investigated sites in wildlife protected areas with different ecological attributes all over Tanzania. These are: Serengeti, Lake Manyara, Tarangire, Ruaha, Udzungwa, Gombe, Mahale and Katavi National Parks as well as Ngorongoro Conservation Area (around Loduare, the main entrance gate). These findings confirm both hypotheses that previously stated that Tanzanian NHPs other than baboons are infected with *T. pallidum* and the infection is not restricted to the northern parts of the country only (Journal Paper I).

The *T. pallidum* infection was not detected in NHPs in Arusha National Park, Ngorongoro Crater, Selous Game Reserve and Jozani-Chwaka Bay National Park in Zanzibar (including one vervet monkey from Masingini forest). The failure to detect TP infection in these areas might be an influence of small sample sizes rather than real absence of the disease in the NHPs living in those locations. However, this study aimed at detecting the presence rather than absence of the disease, which requires comparatively bigger sample size. Similarly, the overall sample size could not allow for strong conclusive remarks on *T. pallidum* prevalence in NHPs at any of the sampled sites. This is due bias in favour of animals that had skin lesions. To overcome this challenge, more objective analysis of the dataset was narrowed down to only include *T. pallidum* infected NHPs that clinically had no any ano-genital or oro-facial skin ulcerations. It was clearly observed that healthy-

looking olive baboons of Lake Manyara National Park and yellow baboons of Mikumi National Park were significantly more often *T. pallidum*–positive than any other NHP species in other sampled sites where those species are present ($p<0.0001$). Again, significant association was observed between presence of antibodies against *T. pallidum* and skin ulcerations only in olive (OR=19.95, $p<0.0001$) and yellow baboons (OR=11.04, $p<0.0185$) but not other NHP species (Journal Paper I).

TPE isolates from Tanzanian NHPs were genetically diverse; however, all of them were closely related to human yaws-causing TPE strains with which they clustered together (Paper I and II). MLST of the TPEs in this study clearly separated them from the TEN strain Bosnia A and syphilis-causing *TPA* strains. MLST analysis showed that interspecies transmission of TPEs likely occurs among Tanzanian NHPs albeit rare (Paper II). This inference is supported by the observed geographical clustering of simian *TPE* strains, regardless of their host species and recovery of multiple TPE strains from the three individual NHPs. The latter also supports possibility of genetic recombination events reported in *T. pallidum* bacteria that, among others, influence infectivity and modulate drug resistance under natural conditions. However, cautious interpretation is needed as the rare interspecies transmission of TPEs among NHPs inferred by this study does not mean spillover to humans.

The relative temporal stability of the isolated simian TPEs strains and absence of antibiotic resistance in Tanzanian NHPS were also demonstrated (Journal Paper II). Based on number of segments with high nucleotide variation in the TP0548 loci, a new difference between TPE strains from NHPs, TEN and TPA from humans is reported by the current study. Based on the newly proposed typing scheme developed by this study, which

involves concatenation of three genes (TP0619, TP0488 and TP0548), a total of 31 new TPE strains have been described and their representative sequences uploaded onto the online database (Tables S3 and S4) (Journal Paper II). Therefore, this study has demonstrated that the MLST technique is a powerful tool in analyzing invasively collected NHP samples from Tanzania and Ethiopia as well as those non-invasively collected from the DRC.

Serological analysis in this study revealed that majority of the NHPs (47.9-98.6%) had antibodies reactive or cross reactive with full viral antigens from viruses of human health relevance (Manuscript). Highly detected antibodies reacted or cross-reacted with: Measles virus (although not detected by other specific confirmatory tests), mouse rotavirus, H1N1 Singapore, minute virus of mice, tick-borne encephalitis virus, mouse hepatitis virus, adenovirus type 3 and yellow fever virus. Other antibodies that reacted or cross-reacted at levels lower than 25% were: Sendai virus, parainfluenza virus type 2, dengue virus, cocksakievirus A24, Sindbis virus, La Crosse virus, Crimean-Congo haemorrhagic fever virus, cowpox virus, rift valley fever virus, hantavirus and human coronavirus 229E.

Presence of antibodies against the detected viruses in NHPs or other genetically or antigenically closely related viruses could neither be linked to *T. pallidum* infection nor presence of ano-genital and/ oro-facial lesions. Therefore, the currently detected viruses and simian TPEs seem to independently infect free-ranging Tanzanian NHPs. None of the NHPs had antibodies reactive or cross reactive with Ebola, Hantavirus (DBOV), Crimean-Congo Haemorrhagic Fever (CCHF-GPC), Rift Valley Fever (RVF), La Crosse (LACV), Sendai (SeV) and Sindbis (SINV) virus antigens, showing that the investigated Tanzanian NHPs had not been exposed to these viruses (Manuscript).

6.2 Recommendations

1. *T. pallidum* infection in NHPs is widespread in NHPs in Tanzania as is elsewhere in Africa and causes very unsightly anogenital and/ orofacial lesions of animal welfare concerns. The disease causes fears for zoonotic transmission among visitors and interferes with their good experience; hence, negatively impacting tourism, to some extent. Tanzania government, through wildlife conservation authorities namely Tanzania National Parks (TANAPA), Ngorongoro Conservation Area Authority (NCAA), Tanzania Wildlife Management Authority (TAWA) and Tanzania Wildlife Research Institute (TAWIRI), is advised to be careful in control and eradicating this disease. For a widespread infectious disease like this, mass treatment is not the best option due to high costs of immobilizing drugs, challenging chemical capture of NHPs, difficult oral delivery of drugs to cover all targeted NHP groups and possible reinfection of the treated NHPs. Treatment of *T. pallidum*-infected NHPs currently on-going at Gombe National Park need to be very cautiously done with regular sampling and analysis to check the TPE isolates for antibiotic resistance.
2. Additional epidemiological studies to improve our understanding of spatial and temporal distribution of simian and human *T. pallidum* infection in Tanzania, East Africa and other parts within and outside the African continent will be very helpful. To start with, investigation of *T. pallidum* infection in NHPs and humans in African countries that share borders with Tanzania is of special interest. Advanced genetic and genomic studies to characterize *T. pallidum* strains from NHPs and humans are critically needed. These studies will further help establishing links between simian *T. pallidum* isolates from NHPs and their counterparts from humans. More attention should be paid to determining whether NHPs act as reservoirs for human infection or vice versa. For sustainable worldwide campaign to eradicate human yaws, the One

Health approach is the most suitable approach for animal and human health research and routine practices at it takes on board animal, humans and environmental health,.

3. All the known *T. pallidum* typing schemes were not suitable and could not type the simian TPE isolates from Tanzania. These include the CDC typing system for *TPA*, the enhanced typing system (Marra *et al*., 2010) successfully used previously for human *TPE* strains (Katz *et al*., 2018), and the recently published alternative method for MLST using *TP0548* locus and two additional loci located in *Tp0136* and *Tp0326* genes (Gordones *et al*., 2017). Whether this is a characteristic of NHP-infecting TPE strains or a spatial property of Tanzanian TPE strains remains unknown. Among significant achievements of the current study is proposing the new typing scheme for TPEs based on the analysis of Tp0548, Tp0488 and Tp0619 genes. Development of a successful global strain typing system for TPE requires a comprehensive database of high-quality genomes obtained from larger numbers of clinical samples from yaws endemic areas. Therefore, efforts be continued towards testing usefulness of the new typing scheme proposed by the current study, fine-tuning the existing scheme(s) and establishing new ones.
4. Pathogenic *T. pallidum* species cannot be grown or propagated on artificial media despite good progress attained in recent research developments. For TPA, *in vivo* inoculation into rabbits and hamsters has been used to propagate these pathogens for further genetic studies and characterizations. It should also be noted that efforts to inoculate simian TPE isolates *in vivo* into New Zealand rabbits did not bear fruits in this study despite of stringently following all the required procedures for inoculation and cultivation of TPA. More studies are needed to establish how best TPE isolates from NHPs in Tanzania and elsewhere in the world, especially Africa, can successfully be *in vivo* inoculated and cultivated into rabbits and hamsters. This is

critically important for propagation of simian TPE isolates and strains for further studies and future uses.

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Appendix 1: Springer Nature's acceptance letter for publication of the Manuscript I

SPRINGER NATURE

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March 27th, 2019

Letter of Acceptance

Dear Sascha Knauf,

I'm pleased to confirm that your contribution **Pathogenic spirochetes in monkeys: stealthy pathogens of global importance**, co-authored by Idrissa S. Chuma, Lena Abel, Luisa K. Hallmaier-Wacker and David Šmajs, was accepted for publication in the book *Knauf & Jones-Engel (eds), Neglected Diseases in Monkeys - From the Monkey-Human Interface to One Health*. The book is currently in preparation; its publication is planned for Q1 2020.

With best wishes,
Silvia Herold

--
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Appendix 2: Immobilization and sampling form used for NHPs studied

Immobilization Form and Sample List

Baboon Disease Research Project

Animal ID#: _____; Pic #1: _____; Pic #2: _____;

Investigator/Gunner: _____; Date: _____.20____;

Species: ⁞ *P.a.* ⁞ *P.c.* ⁞ *C.m.* ⁞ *C.p.* ⁞ *C.an.* ⁞ *C.g.* ⁞ *P.k.* ⁞ *P.g.* ⁞ *P.r.*

⁞ *C.s.* ⁞ *L.u.* ⁞ *R.k.* ⁞ *E.p.* ⁞ *C.as.* ⁞ *P.t.* ⁞ _____;

Group:_____; Location of capture (pref. GPS coordinates): _____;

Longitude _____._____, Latitude _____._____;

Terrain: _____; Troop size: _____ ; Ambient temp. (°C): ___;

Immobilization drugs used

drug: *Ketamin*; dosage used: _____ mg/ kg KM; Vol.: _____ml; applic. modus : IM;

drug: *Xylazin*; dosage used: _____ mg/ kg KM; Vol.: _____ml; applic. modus : IM;

drug: *Atropin*; dosage used: _____ mg/ kg KM; Vol.: _____ml; applic. modus : IM;

drug: *Zoletil*; dosage used: _____ mg/ kg KM; Vol.: _____ml; applic. modus : IM;

drug: *Medetomidine*; dosage used: _____ mg/ kg KM; Vol.: _____ml; applic. modus : IM;

Behaviour at time of injection: (run, walk, stand, etc.) _____;

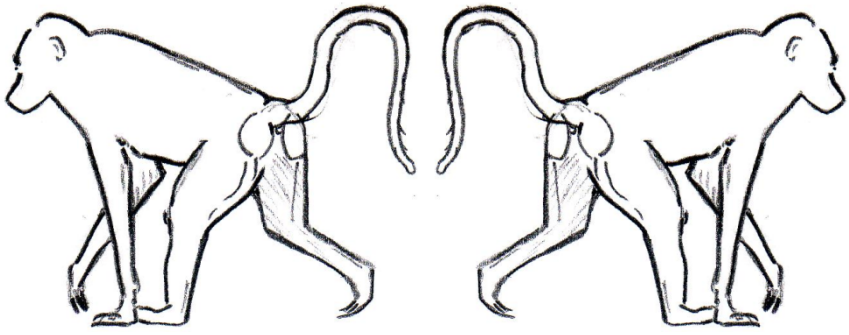
Applic. mod.: ⁞ Blowpipe ⁞ Rifle; Pressure (bar): ____ Distance (m): _____;

First time of injection: _____;

Time of following injection: _____; Reason: _____;

drug: *Ketamin*; dosage used: _____ mg/ kg KM; Vol.: _____ml; administration route: IM;

drug: *Zoletil*; dosage used: _____ mg/ kg KM; Vol.: _____ml; administration route: IM;



Injection side (please mark)

Time of initial effect (ataxia and first signs of sedation): _____;

Time when animal goes down:_____;

Time when down (head down): _____;

Time when arrive at immob. baboon: _____;

How many darts failed, drugs lost:_____;

Signalement

Body Condition Score (0-5): ____; Body weight:_____ kg; male ⁞ female ⁞, female lactating Y ⁞ N ⁞;

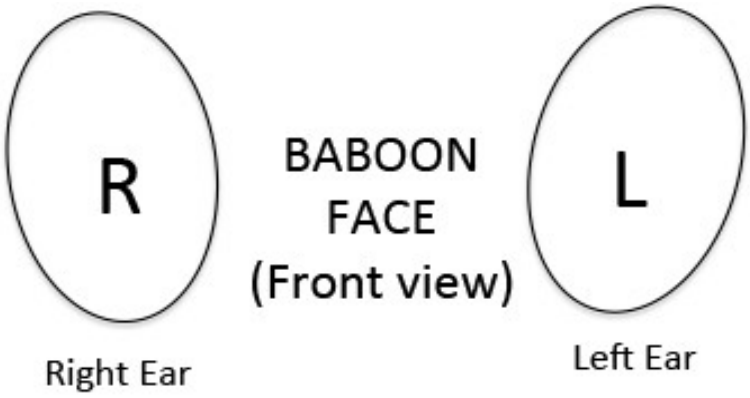
age: _____; permanent incisors: Y ⁞ N ⁞; Emergency treatment: _____;

MUAC (cm) _____; Crown length (cm) _____;

Heart girth: _____; Tail length (cm): _____;

Temporarily identification : (i.e. haircut tail plus silver spray)_____

Permanent identification: (Ear notches) #:_____;



Ear notch sides (please mark)

Vital parameters

Time	SPO_2	HR	RR	Temp (°C)	Remarks

Other vitals:_____;

Clinical examination

Skin and hair: _____ ☐ NAD

Mucosa: _____ ☐ NAD

Lymphnodes: _____ ☐ NAD

Cardio-vascular-system: _____ ☐ NAD

Respiratory system: _____ ☐ NAD

Digestive tract: _____ ☐ NAD

Urinary system: _____ ☐ NAD

Reproductive and genital system: _____ ☐ NAD

Others_____;

Reversal time of immobilisation

Reversal time : _____ Recovery time: _____;

Drug: _____ ; dosage used: _____ mg/ kg KM; Vol.: _____ml; applic. modus : IM;

Additional drugs: **(No Antibiotics and Silverspray for Project #2!!!)**

Drug: *Duphamox* ; dosage used: _____ mg/ kg KM; Vol.: _____ml; applic. modus : IM;

Drug: _____; dosage used: _____ mg/ kg KM; Vol.: _____ml; applic. modus : IM;

General comment to anaesthesia: _____;

Sampling

K_3^+-EDTA blood (min. 2 x 9 ml): quantity _____ ID# _____ ☐

1. Plasma (flash frozen, cryo) quantity _____ ID# _____ ☐
2. Plasma (frozen) quantity _____ ID# _____ ☐
3. Plasma (ni-FTA[1]) quantity _____ ID# _____ ☐
4. Buffy coat (frozen) quantity _____ ID# _____ ☐
5. Buffy coat (ni-FTA) quantity _____ ID# _____ ☐
6. RBCs (frozen) quantity _____ ID# _____ ☐
7. Whole blood (ni-FTA) quantity _____ ID# _____ ☐
8. Blood smear quantity _____ ID# _____ ☐

Lithium Heparin blood (min. 1 x 9 ml): quantity ____ ID# _____ ☐

1. Plasma (frozen) quantity _____ ID# _____ ☐
2. Buffy coat (frozen) quantity _____ ID# _____ ☐

Serum (min. 2 x 9 ml): quantity _____ ID# _____ ☐

1. Serum (frozen): quantity _____ ID# _____ ☐

Cotton Swabs

Lesion (min. 3): quantity _____ ID# _____ ☐

1. Frozen (native) quantity _____ ID# _____ ☐
2. Flash frozen, (cryo, viability prot.) quantity _____ ID# _____ ☐
3. Lysis buffer quantity _____ ID# _____ ☐
4. i-FTA[2] Card (native) quantity _____ ID# _____ ☐
5. RNA-later quantity _____ ID# _____ ☐

Vagina (min. 2): quantity _____ ID# _____ ☐

1. Frozen (native) quantity _____ ID# _____; ☐
2. RNA-later quantity _____ ID# _____; ☐
3. i-FTA Card (native) quantity _____ ID# _____; ☐

Mouth (min. 1): quantity _____ ID# _____ ☐

1. RNA-later quantity _____ ID# _____; ☐
2. Frozen (native, -20°C) quantity _____ ID# _____; ☐
3. i-FTA Card (native) quantity _____ ID# _____; ☐

Pharynx (min. 1): quantity _____ ID# _____ ☐

1. Frozen (native) quantity _____ ID# _____; ☐
2. RNA-later quantity _____ ID# _____; ☐

---

1 Ni-FTA = Non-indicating FTA Card

2 i-FTA = indicating FTA Card

3. i-FTA Card (native) quantity _____ ID# _____; ☐

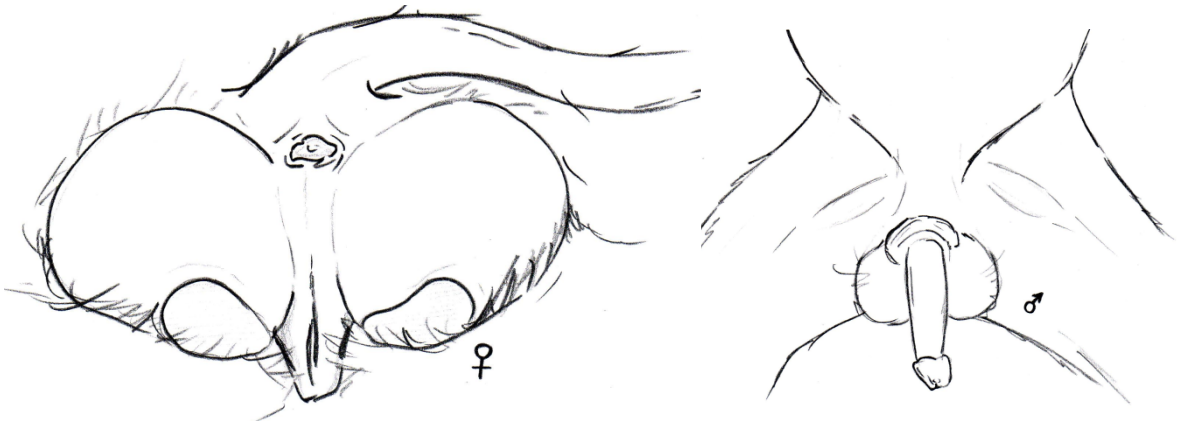
Faeces (min. 3): quantity _____ ID# _____ ☐

1. Frozen (native, -20°C) quantity _____ ID# _____; ☐
2. RNA-later quantity _____ ID# _____; ☐
3. Ethanol >96% quantity _____ ID# _____; ☐
4. Formalin ☐ 4% ☐ 10% quantity _____ ID# _____; ☐

Lymph node (min. 2): quantity _____ ID# _____ ☐

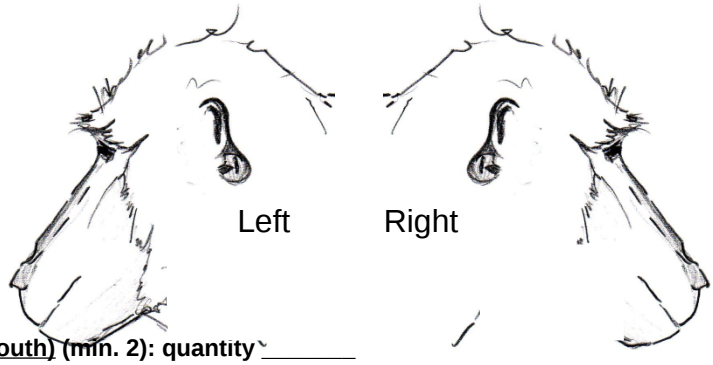
1. Lysis buffer No. 1 quantity _____ ID# _____; ☐
2. Lysis buffer No. 1 quantity _____ ID# _____; ☐

<u>Skin biopsy</u> (min. 4 per animal): ☐



(please indicate biopsy location and lesion)

1. ID# _____; Flash frozen, (<u>cryo, viability protected</u>) ☐ 3. ID# _____; (Frozen) ☐
2. ID# _____; Flash frozen, (<u>cryo, viability protected</u>) ☐ 4. ID# _____; (RNA-Later) ☐
5. ID# _____; (RNA-Later) ☐



<u>CEP SWAB (Mucosa mouth)</u> (min. 2): quantity _____ ID# _____ ☐

1. Lysis buffer No. 1 quantity _____ ☐ ID# _____;
1. Lysis buffer No. 2 quantity _____ ☐ ID# _____;

ESPLINE TP ☐ Serum ☐ Lithium-Heparin Plasma ☐ K_3-EDTA Plasma;

Reference bar ☐ positive ☐ negative

Testresult ⁋ positive ⁋ negative; Pic #3: _____;

other comments

Team

Veterinarian:
Ranger:
Driver:
Assistant:
Accompanying persons: