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Epidemic of venereal treponematosis in wild monkeys: a paradigm for syphilis origin

Oleg MEDIANNIKOV[1,2], Florence FENOLLAR[2,3], Bernard DAVOUST[1,2], Nadia AMANZOUGAGHENE[1,2], Hubert LEPIDI[1,2], Jean-Pierre ARZOUNI[1,2], Georges DIATTA[4], Cheikh SOKHNA[2,3], Jeremy DELERCE[1,2], Anthony LEVASSEUR[1,2], Didier RAOULT[1,2*]

[1] Aix Marseille Univ, IRD, AP-HM, MEΦI, Marseille, France

[2] IHU-Méditerranée Infection, Marseille, France

[3] Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France

[4] VITROME, Campus International UCAD-IRD, Dakar, Senegal

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[*]Corresponding author: Didier RAOULT

Address: Institut Hospitalo-Universitaire - Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille, France

Phone: +33413732401; Fax: +33413732402; e-mail: didier.raoult@gmail.com

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Author contributions

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ABSTRACT

Background

Treponema pallidum infections have been primarily known as slightly contagious mucocutaneous infections, called yaws (tropical Africa and America) and bejel (subtropical North Africa). *T. pallidum* emerged as a highly infectious venereal syphilis agent in South America, probably about 500 years ago, and because of its venereal transmission, quickly caused a worldwide pandemic. The disease presents three stages: lesions including a chancre, then antibodies become detectable when or slightly after the chancre appears and prior to the development of the rash and other systemic manifestations. Venereal diseases were previously poorly known in monkeys.

Methodology/Principal Findings

During a fieldwork in Senegal, we discovered an epizootic outbreak of venereal disease that we explored. We detected a venereal form of *T. pallidum pertenue* infection in green monkeys and observed an epizootic outbreak in Senegal during one year, and its spread among baboons. Comparative analysis of *T. pallidum* genomes from the monkeys' chancres and other *Treponema* genomes shows an acceleration of SNPs number comparable to that observed in syphilis.

Conclusions/Significance

Identified *T. pallidum* clones seem to be epizootic through the mutation rate acceleration linked to their larger diffusion.

AUTHOR SUMMARY

All three subspecies of *Treponema pallidum* are pathogenic for humans: *T. p. pallidum* causes syphilis, *T. p. endemicum*, causes bejel or endemic syphilis and *T. p. pertenue* which causes yaws. The relationship among these species and their natural history is the subject of discussions. It is generally thought that about 500 years ago, *T. pallidum* has emerged as a highly infectious venereal syphilis agent in South America and quickly caused a worldwide pandemic because of its venereal transmission.

During a fieldwork in Senegal, we discovered an outbreak of venereal disease in green monkeys. We detected a venereal form of *T. pallidum pertenue* infection in green monkeys. In one year, we also observed the spread of the infection to all green monkey population and its dissemination among baboons. Comparative analysis of *T. pallidum* genomes from the monkeys' chancres and other *Treponema* genomes shows an acceleration of SNPs number comparable to that observed in syphilis. We believe that we evidenced a developing epizootic outbreak in monkeys caused by a specific clone of *T. pallidum* . It may be linked to the mutation rate acceleration.

INTRODUCTION

Treponema pallidum infections have been primarily presented as slightly contagious mucocutaneous infections, called yaws (tropical Africa and America) and bejel (subtropical North Africa). Overall, the origin of treponematoses and relations between the pandemic venereal syphilis and endemic treponematoses (yaws, bejel, and pinta) are not yet clear and even palaeontological evidences are not confirmative (1). Although endemic treponematoses have undergone a mass eradication campaign in the 1950-1960 (2), now, all treponematoses are coming back (3) and there is an urgent need to understand the pathogens that cause these diseases in order to eradicate them.

Moreover, treponemes cannot easily be cultured in traditional laboratory settings (4;5) and this is one of the reasons why we know little about these bacteria. Indeed, comparative genomics is one of the means to understand the biology of these bacteria better.

Whole genome sequencing of agents of syphilis (6), yaws (7), and bejel (8), showed very subtle differences (mostly, single nucleotide polymorphism) among these agents with almost the same genome structure and less than 0.2% divergence (7). The current dominating theory is that syphilis appeared as a venereal epidemic caused by a clone derived from the endemic yaws agent, *Treponema pallidum pertenue*, in pre-Columbian America (9) and quickly caused a worldwide pandemic following Columbus's discovery.

First evidences of endemic *T. pallidum* monkey infection were reported from Guinea baboons *Papio papio* (without genital lesions) in Senegal and Guinea (10-13) using serology. An isolate (Fribourg-Blanc) was also obtained from a popliteal lymph node. This strain is pathogenic for hamsters (12) and humans (14), and is genetically very similar to human yaws (15). Since then, monkeys and apes were found to suffer from endemic treponematoses comparable to yaws (16). Genital lesions caused by *T. pallidum* in non-human primates including *Papio anubis, Papio cynocephalus, Chlorocebus pygerythrus,* and *Cercopithecus mitis* were reported in Tanzania (12;17-19) suggesting sexual transmission. Serological

evidences of treponemal infection without lesions were also reported in *Papio papio* in Senegal in 2013-2014 (18) and in Tanzania (19;20).

During a fieldwork in Senegal, for the collection of monkey specimens originally planned for a study on Simian immunodeficiency viruses (SIV), we discovered by chance an epidemic of venereal disease that we explored. Thus, we report herein the first observed epidemic of venereal treponematosis in wild monkeys.

MATERIALS AND METHODS

Study population

Green monkeys and baboons were studied in two different natural parks in Senegal (West Africa). The Niokolo-Koba national park is a World Heritage Site and one of the oldest natural protected areas in Africa. It is situated in southeastern Senegal near the Guinean border. The reserve of Bandia is a small private natural reserve situated 65 km south of Dakar, the capital of Senegal. One population in each park was studied in 2015: the green monkeys group living in the proximity of the Welcome center of Bandia natural reserve (14°33'35.5"N 17°00'30.6"W) and the group of monkeys in the Niokolo forestry guardhouse in Niokolo-Koba national park (13°04'28.6"N 12°43'18.2"W). In 2016 in Niokolo-Koba national park we studied the same population of green monkeys in Niokolo forestry guardhouse as well as Guinea baboons living in sympatry and another population of green monkey from Simenti site (13°01'34.5"N 13°17'40.9"W).

Ethics Statement

The study was approved by the Senegalese Ministry of the Environment (Direction of the National Parks, number 1302, October, 16, 2015 and number 904, July, 12, 2016) and performed in accordance with the recommendations of the Weatherall report.

Sample collection

Three clinically healthy male green monkeys were captured in the reserve of Bandia and 13 (11 males and 2 females) in Niokolo-Koba national park in November 2015. Among

90 animals collected in Niokolo-Koba national park, four male monkeys presented chancre-like
91 lesions on the penis. None of the two female green monkeys showed clinically evident
92 lesions. Animals were not tagged. In 2016, 21 green monkeys (including 14 male and 7
93 female monkeys) were captured in Niokolo-Koba (two populations); among male monkeys,
94 four presented chancres. Besides, among 14 Guinea baboons (13 male and 1 female) living in
95 sympatry; 6 males presented with chancres (Supplementary Table 1). All individuals were
96 anaesthetized using ketamine (10 mg/kg), which was administered intramuscularly via
97 blowpipe or tranquilizer gun.
98 Cotton swab (Copan Diagnostics, Murrieta, California, USA) samples were taken from
99 the edge of a lesion in clinically affected individuals and from normal unaffected tissues in
100 healthy individuals and immediately frozen in liquid nitrogen. Anal swabs were taken from all
101 monkeys. Blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes from the
102 femoral vein. Plasma and erythrocytes were separated by sedimentation during 3 hours and
103 then frozen in liquid nitrogen. Samples were exported to France at -20°C and then transferred
104 to a -80°C freezer. A tissue biopsy was took from the chancre-like lesion of the penis of one
105 baboon.
106 ***Histologic analysis and immunohistochemical detection of T. pallidum***
107 Formalin-fixed, paraffin-embedded tissue specimen obtained from baboon was cut to 3
108 µm in thickness and stained with routine haematoxylin and eosin (H&E) staining. In order to
109 detect the spirochetes, serial sections were also obtained for Warthin-Starry staining and
110 immunohistochemical investigations. The immunohistochemical procedure used a rabbit
111 polyclonal antibody specifically directed against *T. pallidum* diluted 1:100 as recommended
112 by the manufacturer (Biocare Medical, Pacheco, CA, USA) with the Ventana Benchmark
113 autostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA). A negative control was
114 performed using an irrelevant rabbit polyclonal antibody.
115 ***Western-blotting***

The Euroimmun anti-*T. pallidum* Western-blotting tests (Euroimmun, Lübeck, Germany) were performed according to the manufacturer's instructions. Samples and negative controls (human sera previously tested negative for syphilis) were diluted in serum diluent (1:51), and 1.5 mL of the diluted samples was dispensed in each channel with a strip. After incubation for 30 minutes on a rocking shaker at room temperature, the diluted samples were aspirated off and washed 3 times for 5 minutes with 1.5 mL of diluted universal buffer. Strips were then incubated with 1.5 mL of the enzyme conjugate (alkaline phosphatase conjugated antihuman IgG) solution for 30 minutes with agitation, pipetted out and washed 3 times for 5 minutes with 1.5 mL of diluted buffer. Strips were further incubated on a rocking shaker with 1.5 mL of substrate solution for 10 minutes at room temperature. The substrate solution was pipetted out, and strips were rinsed 3 times with cold deionised or distilled water and air-dried.

The development of colored bands on a strip indicated the presence of anti *T. pallidum* IgG antibodies. Strips with these bands were compared against a positive control strip with bands identifying TpN15, TpN17, p22 (unspecific), TpN45, and TpN47 protein antigens. A sample was considered positive if the test strip displayed more than one of the 4 specific bands (TpN15, TpN17, TpN45, and TpN47).

Molecular detection

The genomic DNA was extracted from cotton swabs by using a QIAamp Tissue Kit (Qiagen, Hilden, Germany) with the EZ1 apparatus following the manufacturer's protocols. The genomic DNA was stored at -20°C under sterile conditions until the next stage of the investigation. DNA was stored at −20°C until assayed by PCR. The qPCR assays were performed to screen all samples using *polA*-based and *flaA*-based primers and probes (Supplementary Table 1) (21;22). All qPCRs were performed using a CFX96™ Real-Time system (Bio-Rad Laboratories, Marnes-La-Coquette, France) and the Eurogentec Master Mix Probe PCR kit (Eurogentec, Seraing, Belgium). We included *T. pallidum* DNA extracted from

a syphilis patient's swab as positive control and master mixtures as a negative control for each assay. Samples were considered positive when the cycle's threshold (Ct) was lower than 35 Ct. In order to identify the subspecies of *T. pallidum* infecting monkeys, we chose two genes (TENDBA_0136 and TENDBA_0859 based on *T. pallidum endemicum* genome) (8) that would enable us to characterize identified treponema (Supplementary Table 1).

Treponemal genome sequencing by in-solution hybridization capture

DNA extracts were fragmented using a Covaris S220 Focused-ultrasonicator® in a volume of 130 µl low EDTA TE buffer using settings to generate a modal 400 bp fragment size (Intensity = 4, Duty cycle = 10%, Cycles per burst = 200, Treatment time = 55 s, Temperature = 7°C). Fragmented extracts were concentrated using the MinElute PCR purification kit and eluted into 2x10ul low EDTA TE buffer (Qiagen). DNA concentrations of all extracts were then measured using a Qubit dsDNA High Sensitivity kit (Thermo Fischer Scientific, Waltham, MA, USA). Libraries were prepared using the Accel-NGS 2S DNA PCR free library kit following the standard protocol, with unique single indices (Swift Biosciences, Ann Arbor, MI, USA). Libraries were quantified using the KAPA HiFI library quantification kit (Roche, Basel, Switzerland) and, when needed, subsequently amplified using the KAPA Hot Start Library Amplification Kit (Roche), with Illumina adaptor specific primers, and then re-quantified. Samples were pooled according to species: i) for baboon libraries, 100 ng per library was used (total pool mass: 700 ng), ii) and for African green monkey libraries, 140 ng per library was used (total pool mass: 700 ng).

RNA baits covering the entire *T. pallidum pertenue* Fribourg-Blanc genome were designed with 2-fold tiling of 120mer baits. We essentially followed the myBaits Sequence Enrichment for Targeted Sequencing protocol (Version 2.3.1) but only used 125 ng bait per capture round and a hybridization time of 20 hours (Arbor Biosciences, Ann Arbor, MI). Following this first round of capture, the capture product was amplified using the KAPA Hot Start Library Amplification Kit for 20 cycles. DNA was concentrated using a MinElute PCR

Purification Kit and 1 µg of DNA was used for the second round of hybridization capture, performed under the same conditions as for the first round of capture. The final capture product was amplified using the KAPA Hot Start Library Amplification Kit for 12 cycles, purified using the MinElute PCR Purification Kit, quantified using the KAPA HiFi Library Quantification Kit, and diluted to 4 nM as input for an Illumina MiSeq run (v3; 2x300 bp).

Bioinformatic and evolutionary analyses

The draft genome sequences of *T. pallidum* A10, *T. pallidum* A12, *T. pallidum* BNK07, and *T. pallidum* SNK05 have been deposited at European Bioinformatics Institute (EBI), European Nucleotide Archive (ENA) under the accession numbers: OMPM01000001-OMPM01000006; OMHU01000001-OMHU01000103, OMHW01000001-OMHW01000017, OMHZ01000001-OMHZ01000216, respectively.

All other *Treponema* genomes (CP002103, CP016062, CP016065, CP010422, CP007548, CP002375, CP002376, and CP003902) were downloaded from NCBI (www.ncbi.nlm.nih.gov). After sequencing, reads from BNK07, A10, and A12 were mapped on the reference *T. pallidum* Fribourg Blanc using CLC Genomics workbench 7 with the following thresholds: 0.7 for coverage and 0.8 for similarity. Consensus sequences were extracted from the mapped reads (>3 reads threshold).

As for single nucleotide polymorphisms (SNPs), SNPs were calculated using CLC Genomics workbench 7 based on consensus sequences. Two hypervariable regions (positions 230-240 kb and 280-290 kb) were excluded from the analyses. Genomes were annotated using Prokka (23) with default parameters. Regarding ortholog detection, we applied Proteinortho with default values (24). All orthologous genes were aligned using Muscle (25), then concatenated. Phylogenetic reconstruction (for Figure 4) was performed using RaxML (Randomized Axelerated Maximum Likelihood) with the GTRCAT model and a bootstrap value of 100 (26).

In order to investigate the possible relationships among strains, the neighbor-joining network using the Bandelt method was constructed using the NETWORK5.0 program(www.fluxus-engineering.com/sharenet.htm) using equal weights for all mutations (27).

RESULTS

In November 2015, in order to study SIV, we investigated and sampled two populations of green monkeys (*Chlorocebus sabaeus*) in Senegal: one from Bandia private nature reserve and one from Niokolo-Koba National park (Figure 1). In Niokolo, we discovered serendipitously typical chancrous lesions looking like human syphilis on four of fourteen monkey penises (Figure 2 and supplementary Figure 1) that were positive for *T. pallidum* using two different specific qPCR assays (Supplementary Tables 1 and 2). Specific Western-blot serology (Figure 2) was also positive for the four symptomatic monkeys. Among the eight asymptomatic monkeys, only three were Western-blot positive, but two females and three juvenile males were negative. All monkeys from Bandia reserve were negative.

During a second mission in August 2016, we observed the development of this venereal disease in various monkey populations, the natural evolution of this venereal disease mimicking primary syphilis in humans. We studied two populations of green monkeys in Niokolo-Koba, including the same population studied in 2016. All captured monkeys were seropositive in both populations, with a significantly higher prevalence than in 2015 (7/12 in 2015 versus 21/21 in 2016, Fisher exact test =0.003, $p < 0.05$). Moreover, five out of twenty-one monkeys were qPCR-positive: three males with chancre-like genital lesions (genital swabbing), one female without lesions (genital swabbing), and one male without genital lesions (anal swabbing) (Supplementary Figure 1). Although animals captured in 2015 were not tagged, serendipitously, in 2016 we captured remarkable one-eyed male, the same already tested one year ago. His chancre spontaneously regressed to a qPCR-negative scar (Supplementary Figure 1), mimicking the natural history of primary syphilis in humans.

In August 2016, we also captured fourteen Guinea baboons living in sympatry with green monkeys. We detected penis chancres on six baboons (Supplementary Figure 1), all qPCR-positive. Moreover, six out of eleven sampled sera were Western-blot positive including three without genital lesions. One PCR-positive monkey was seronegative, suggesting very recent infection. Spirochetes were stained in great number in the connective tissue of the foreskin using Warthin-Starry stain and by immunohistochemistry (Figure 2 and Supplementary Figure 2). Bacteria were mixed with an inflammatory infiltrate composed of lymphocytes, plasma cells, and neutrophils (Supplementary Figure 2). All monkeys with genital lesions observed in 2016 were then treated by injections of prolonged penicillin.

Firstly, we selected two gene fragments presenting differences among most known *T. pallidum* subspecies. A 589-bp portion of TENDBA_0136 gene coding for virulence-associated *Treponema* conserved hypothetical proteins that binds human fibronectin (28) was found to be identical for all qPCR-positive monkeys (both green monkeys and baboons). It was also identical to all strains except SamoaF strains of yaws agent and Fribourg-Blanc *Treponema*. A 464-bp portion of TENDBA_0859 gene coding for a protein of unknown function was identical in all green monkeys sampled in 2015 and 2016 but also in one baboon (BNK14, Supplementary Table 2) sampled in 2016 and infected by the same *Treponema* as green monkeys. All other baboons had two identical point mutations differentiating them from all known *Treponema* strains, evidencing the circulation of another clone. Closest identity was, however, with Fribourg-Blanc *Treponema* (462/464) (Supplementary Figure 3).

The clinical natural history and dynamics (2015 to 2016) of the discovered disease resembles that of syphilis: apparition of a chancre harboring *Treponema* before serological response, then the chancre heals spontaneously; finally, anti-treponemal antibodies appear and persist (Figure 2). Here, we are facing an epizootic outbreak of a bacterial infection. Such outbreaks are often caused by a single clone (29-32), whereas endemic diseases are caused by several genotypes. To evaluate the evolutionary ratio of this putative clone we sequenced it.

We succeeded in sequencing almost entirely the genomes of *Treponema* from lesions of baboons (BNK07) and green monkeys (SNK05) sampled in 2016 and from two green monkeys (A10 and A12) sampled in 2015, and compared them to other open source complete genomes. We found that they were nearly identical (Figure 3, Table 1). We have obtained the sequence of the treponeme genome sequences from the geographical region where Fribourg-Blanc isolated his strain in 1966. This strain is thought not to have evolved very much in the laboratory because it remained most of the time frozen since the 1960s. The relationships between different strains of subspecies of *T. pallidum* based on SNPs are also represented on the Median Joining Network (MJN) (Figure 4).

Table 1. Pairwise comparison of the number of SNPs between different strains.

	A10	**A12**	**BNK07**	**SNK05**	***T. pallidum* str Fribourg-Blanc CP003902**	***T. pallidum* subsp *endemicum* str Bosnia CP007548**	***T. pallidum* subsp *pallidum* str PTSIF1200 CP016062**	***T. pallidum* subsp *pallidum* str PTSIF1261 CP016065**	***T. pallidum* subsp *pallidum* str Seattle Nichols**	***T. pallidum* subsp *pertenue* str CDC2 CP002375**	***T. pallidum* subsp *pertenue* str Gauthier CP002376**
A12	53										
BNK07	64	108									
SNK05	112	155	145								
***T. pallidum* str Fribourg-Blanc CP003902**	66	108	63	146							
***T. pallidum* subsp *endemicum* str Bosnia CP007548**	371	397	368	439	372						
***T. pallidum* subsp *pallidum*str PTSIF1200 CP016062**	801	837	797	863	802	848					
***T. pallidum* subsp *pallidum* str PTSIF1261 CP016065**	798	834	790	853	795	842	3				
***T. pallidum* subsp *pallidum* str Seattle Nichols**	830	862	822	884	824	857	167	167			
***T. pallidum* subsp *pertenue* str CDC2 CP002375**	101	145	105	185	94	342	777	769	799		
***T. pallidum* subsp *pertenue* str Gauthier CP002376**	117	158	119	196	111	357	791	781	808	67	
***T. paraluiscuniculi* str Cuniculi CP002103**	6,269	6,225	6,237	6,139	6,28	6,291	6,278	6,281	6,316	6,27	6,268

Strains (str) A10 and A12 are from green monkeys sampled in November 2015, SNK05 from a green monkey sampled in August 2016 and BNK07 from a baboon sampled in August 2016.

Thus, the single nucleotide polymorphism between *Treponema* from BNK07 (found in 2016) baboons and Fribourg-Blanc strain (isolated 50 years ago) in only 63 base pairs may correspond to the natural evolution of the genome of *T. pallidum*, *i. e.*1.26 mutations per year. Interestingly, this is comparable with the generally accepted mutation rate in unicellular eukaryotes and prokaryotes of roughly 1/300 mutations (10^{-6} MB/generation) per effective genome per cell generation (33) applied to one *T. pallidum* generation in 30 hours (34). Moreover, the average number of 2111.7 nucleotide differences between syphilis and yaws strains (7) may be easily obtained by applying this mutation rate for the 500-year-old documented history of treponematoses in the Old World (7;8;15). Therefore, the forgotten and neglected now Hudson's Unitarian hypothesis (35;36), stating that all human treponematoses are caused by the same agent and that differences are due to climatic conditions and social habits of the targeted population, may have a second life. Other authors, however, estimate the evolution time between yaws and syphilis agents in around 10,000 years (37), although the most recent common ancestor of human syphilis strains evolved after 15th century, precisely around 1744 (38). The numbers of SNPs detected between strains isolated during rapidly evolving outbreak in 2015 (A10, A12) and 2016 (SNK05) were 112 and 155, respectively. Although it may be explained by sampling existing genetic variations in this bacterial population, these numbers of SNPs are particularly elevated while only one year separated both samplings and, consequently, reflected the epidemic nature of these clones. The long branch regrouping *Treponema* strains of the current epizooty in monkeys on the phylogenetic tree (Figure 4) also resembles that of the *Treponema* of syphilis.

DISCUSSION

We believe that the emergence of an outbreak of venereal clonal treponematosis in monkeys may be a paradigm of human syphilis. A non-venereal *Treponema* infection due to the agent of human yaws, *T. pallidum pertenue,* is known in monkeys in Western Africa (39). We occasionally identified in 2015 is what we think to be the beginning of the outbreak in

green monkeys of West Senegal. The population of green monkeys in Niokolo-Koba is estimated as 30,000 individuals (40); in Bandia reserve that is situated in about 550 km to the north-west, no estimation is available and may be around several thousands. Within a few months, the tested population of green monkeys in Niokolo-Koba was infected. Moreover, it was found in baboons in which we identified 2 genotypes including one almost identical to that of green monkeys. We think that it is compatible with cross-species transmission of *T. pallidum pertenue* that was already reported in Côte d'Ivoire (41). Although only few green monkey were studied in Bandia reserve, the absence of veneral treponematosis (both acute and serological) may mean that the presumable outbreak didn't yet reach here. We speculate that this outbreak resembles the emergence of syphilis that started as a local venereal disease, stemming from endemic yaws in America, and became rapidly pandemic following Columbus's arrival (42).

The difference between yaws and syphilis is that syphilis is a very contagious, venereal disease, which develops in three stages (43), an inoculation chancre where treponema is found and where antibodies are not found, spontaneous healing of chancre where antibodies are found for life. First, we detected these antibodies in monkeys by Western-blot, and then we have seen a dramatic extension of syphilis in one year from a part of the population to entire population (based on serology). The late clinical manifestations, secondary (rash) and tertiary syphilis (gum, neurological, and vascular involvements) are currently poorly described in monkeys (41;44) and must be investigated. The rash, however, may be invisible in monkeys.

In addition, genome studies show that the bacterium is very close to the agent of yaws (once endemic in Senegal) with a number of SNPs compatible to a rapid and epidemic spread of syphilis observed elsewhere. In addition, we hypothesize the transfer of this clone to baboons (based on identification of a very close strain found in one baboon). It suggests that the disease could spread rapidly to different primates. Thus, this strain of *Treponema* may become epidemic and this outbreak may originate from the emergence of a clone. In

310 treponemes, this has already happened once in humans with the global syphilis epidemic. The
311 genomic era allows a paradigm change. It becomes clear now that in most cases such as here
312 but also for tuberculosis with the Beijing clone (29), with *Clostridium difficile* clones (30),
313 with typhoid (31) or with cholera (32), the epidemics are caused by clones emerging among
314 numerous endemic strains following usually little genetic modifications. Currently, genomic
315 studies carried out on these bacteria have made it possible to show, with respect to endemic
316 strains, an increase in the number of SNPs which makes possible to identify the epidemic
317 clones because their multiplication number is greater than that of the endemic species. We
318 believe that we evidenced the beginning of an epizooty of treponematosis. Its risk of
319 dissemination to other populations of monkeys and apes requires an active surveillance in
320 these primate populations.

321 These results lead to at least two important questions regarding emergence of an
322 epidemic of treponematoses: i) If this zoonosis threatens to become the reservoir of a new
323 disease in humans as it has already happened with HIV (45), ii) If prevention could be
324 considered with systematic treatment of monkey populations in which one or more cases were
325 found to be positive, for example by penicillins *per os* in the food.

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Figure legends.

Figure 1. Map of Senegal with the locations of collection sites (Map created from Wikimedia Commons: https://commons.wikimedia.org/wiki/Atlas_of_the_world).

Figure 2. Parallel in the clinical course between human syphilis and treponematosis of green monkeys. The chancre was on the penis of a green monkey (Niokolo forestry guardhouse) sampled in November 2015 (A09) and was PCR positive for *T. pallidum*. The visualization of *T. pallidum* (brown spirochetes) using immunohistochemical detection (original magnification x 400) was based on the biopsy of a penis lesion found on a baboon sampled in August 2016 (BNK14). The positive western blotting serology was obtained from a green monkey sampled in August 2016 (SNK21).

Figure 3. Phylogenomic tree reconstruction based on the core genomes of *Treponema* isolates including *T. pallidum* subspecies and monkey *T. pallidum* from the present study.

Figure 4. Median Joining Network (MJN) representing the relationships between different strains of subspecies of *T. pallidum* based on SNPs. Each circle area indicates a unique genotype. The number of mutation steps between strains is indicated in red.

459 **Supporting Information Legends**

460 **Supplementary Table 1.** List of primers and probes used in the study.

461 **Supplementary Table 2.** Monkeys included in the study from Bandia reserve (BR), Niokolo-
462 Koba National park, Niokolo Forestry Guard (NFG), and Niokolo-Koba National park,
463 Simenti group (SG) as well as results of the detection of treponemes.

464 **Supplementary Figure 1.** Syphilis-like lesions of monkeys from Niokolo-Koba National
465 park, Senegal. (A) Recovering penis lesions, *C. sabaeus*, Niokolo forestry guardhouse,
466 August 2016. (B) Penis and scrotum lesions accompanied by regional lymphadenopathy
467 (arrow), *C. sabaeus*, Simenti, August 2016. (C) Penis and scrotum lesions, *P. papio*, Simenti,
468 August 2016.

469 **Supplementary Figure 2.** Visualization of *T. pallidum* (arrows) in penis lesions in Guinea
470 baboons using Warthin-Starry staining (original magnification x 1,000).

471 **Supplementary Figure 3.** Molecular phylogenetic analysis of *Treponema* strains by
472 Maximum Likelihood method based on concatenated sequences of TENDBA_0136 and
473 TENDBA_0859 genes. The Genbank accession numbers of *Treponema* genomes are
474 indicated at the beginning. The evolutionary history was inferred by using the Maximum
475 Likelihood method based on the HKY model. The bootstrap consensus tree inferred from
476 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed. The
477 analysis involved 23 nucleotide sequences. Positions containing gaps and missing data were
478 eliminated. Evolutionary analyses were conducted in Topali.









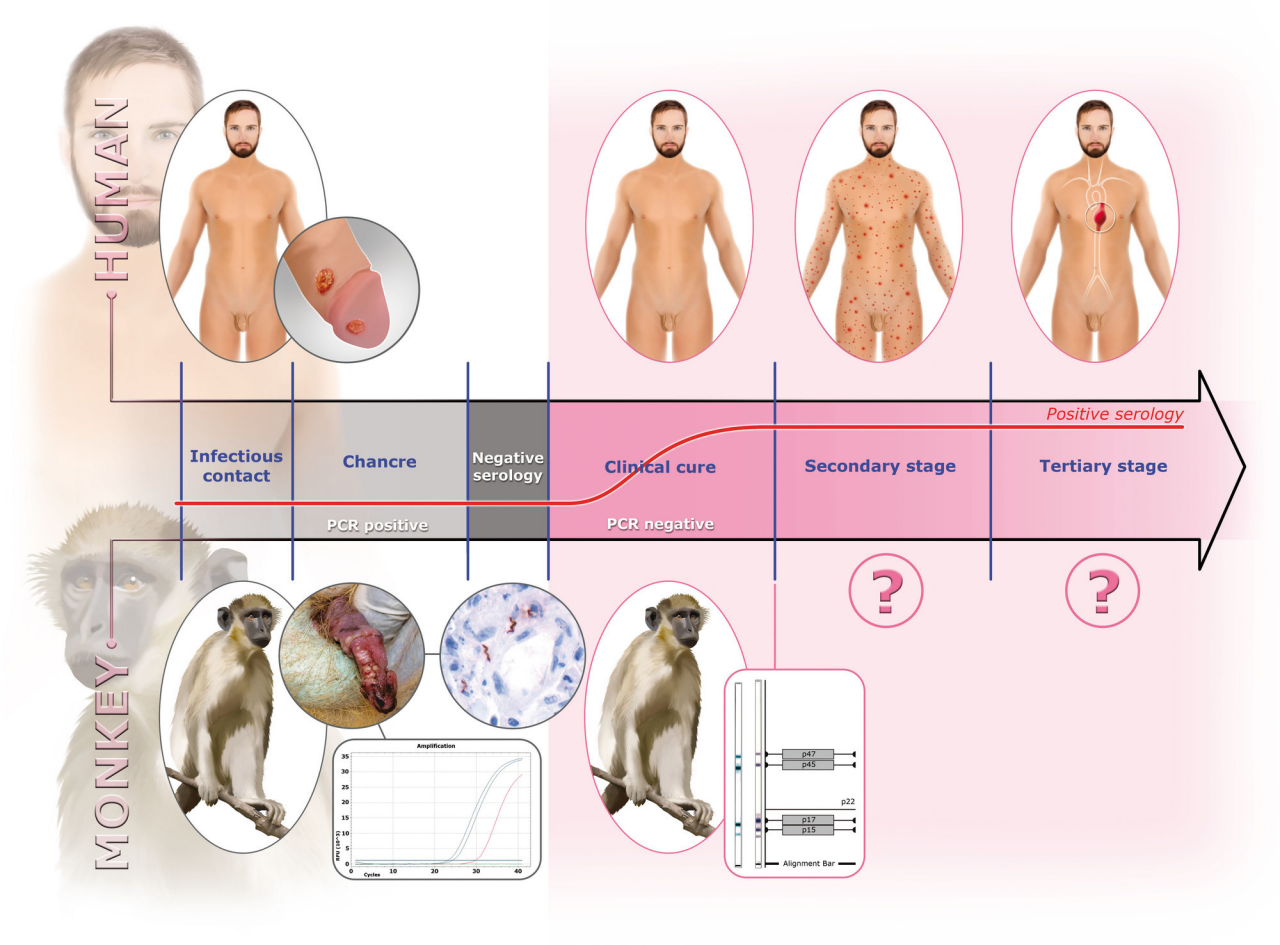
Dakar

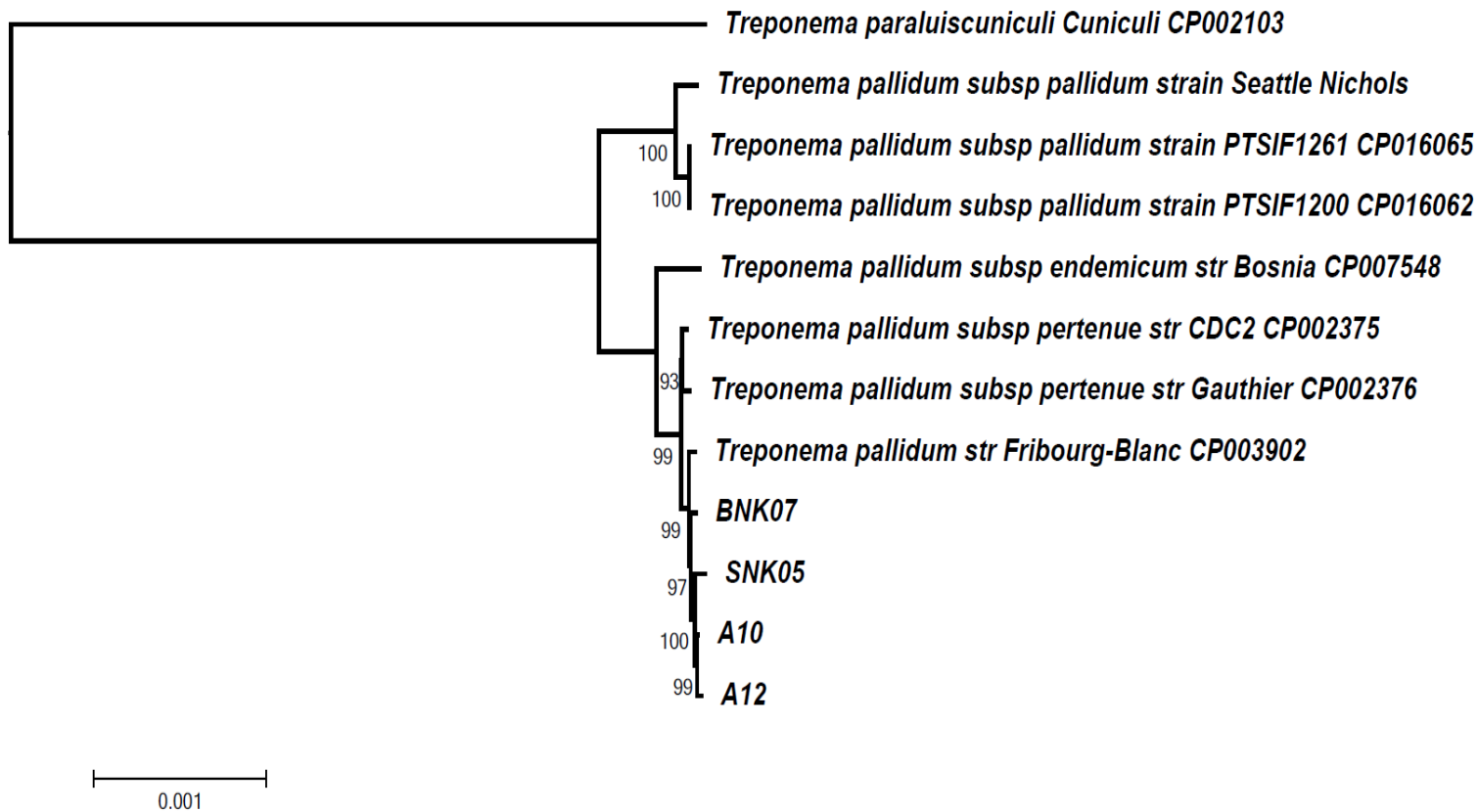
Bandia reserve, 2015

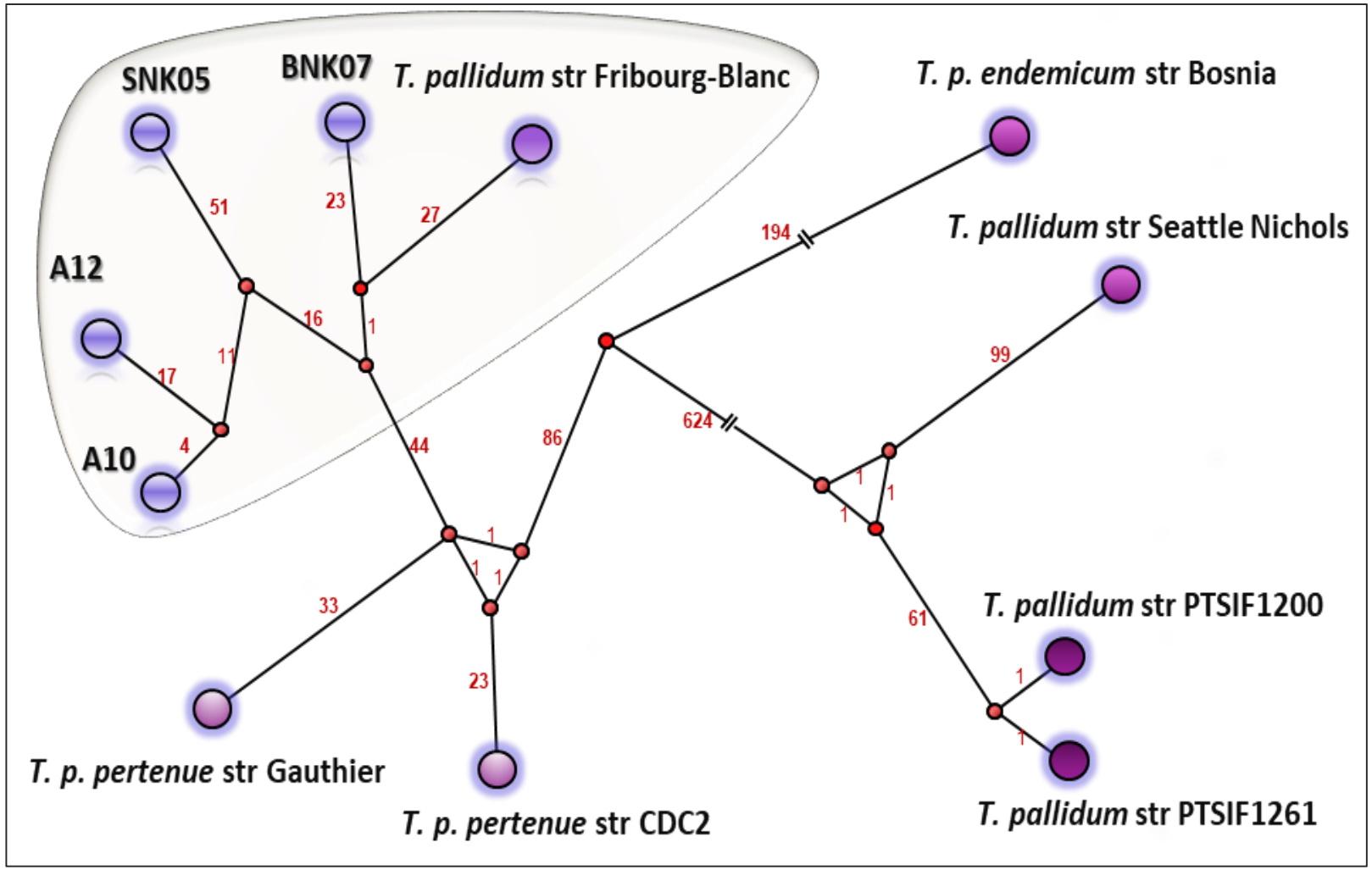
Niokolo-Koba national parc

Forestry guard, 2015 and 2016

Simenti, 2016


HUMAN
MONKEY
Infectious contact
Chancre
Negative serology
Clinical cure
Secondary stage
Tertiary stage
Positive serology
PCR positive
PCR negative
?
?
Amplification
Cycles
p47
p45
p22
p17
p15
Alignment Bar


Treponema paraluiscuniculi Cuniculi CP002103
Treponema pallidum subsp pallidum strain Seattle Nichols
100
Treponema pallidum subsp pallidum strain PTSIF1261 CP016065
100
Treponema pallidum subsp pallidum strain PTSIF1200 CP016062
Treponema pallidum subsp endemicum str Bosnia CP007548
Treponema pallidum subsp pertenue str CDC2 CP002375
93
Treponema pallidum subsp pertenue str Gauthier CP002376
99
Treponema pallidum str Fribourg-Blanc CP003902
BNK07
99
SNK05
97
A10
100
A12
99
0.001



A10 and A12 are from green monkeys sampled in November 2015, SNK05 from a green monkey sampled in November 2015 and BNK07 from a baboon sampled in August 2016.