Severe case of yaws disease caused by *Treponema pallidum* subsp. *pertenue* in a wild chimpanzee (*Pan troglodytes verus*) in Tinguelita, Sangaredi area, Guinea, 2019

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Abstract

Yaws-like lesions are widely reported in wild African great apes, yet the causative agent has not been confirmed in affected individuals. We describe yaws-like lesions in a wild chimpanzee in Guinea for which we demonstrate infection with *Treponema pallidum* subsp. *pertenue*. Assessing the conservation implications of this pathogen requires further research.

Several monkey species in sub-Saharan Africa are infected with *Treponema pallidum* subsp. *pertenue* (TPE) and typically manifest yaws-like lesions on the face and distal extremities or syphilis-like lesions in the anogenital region (*1*). The first reports of NHPs infected with TPE were from West Africa in the 1960s. These were based on sero-prevalence studies finding that yellow baboons (*Papio cynocephalus cynocephalus*) had a 60% sero-prevalence rate for treponemal specific antibodies (*2,3*). Whole genome sequencing of the isolate collected from these baboons later revealed similarities with TPE causing yaws in humans (*3,4*). In the late 1980s in Gombe National Park, Tanzania, olive baboons (*Papio anubis*) with genital ulcerations were found to have yaws-like infections of the skin (*5*). Later genetic and serological studies confirmed infections with *Treponema pallidum* (TP) in olive baboons (*Papio anubis*) at many sites in Tanzania (*6,7*). Both genital and orofacial lesions due to TPE infection have now been documented in a number of NHP species across sub-Saharan Africa (African green monkeys: *Chlorocebus sabaeus* in Bijilo Forest Park, The Gambia and Niokola –Koba National Park, Senegal; sooty mangabeys (*Cercocebus atys atys*) in Taï National Park (TNP), Côte d'Ivoire) (*1,8*). Chuma and co-workers observed that TPE infections remain geographically widespread in Tanzania and affect olive baboons, yellow baboons*,* vervet monkeys (*Chlorocebus pygerythrus*) and blue monkeys (*Cercopithecus mitis),* as well as grivet monkeys (*Chlorocebus aethiops*) from Ethiopia (*9,10*).

Symptoms and skeletal deformation have also been observed in great apes, specifically gorillas (*Gorilla gorilla*) in the Republic of Congo, Gabon, and Cameroon (*11*), as well as chimpanzees (*Pan troglodytes*) in Cameroon, Uganda, and Côte d'Ivoire, and are suggestive of TPE infections (*11*, pers. comm. F. H. Leendertz) but matching diagnostics are currently unavailable. The only diagnostic evidence is based on TPE DNA from two chimpanzee (*Pan troglodytes verus*) bones (*12*) and gorilla feces (*10*) of unknown individual great apes, so no link between diagnostics and clinical signs can be made. Here we present matching clinical and molecular evidence of TPE infection in a wild great ape.

The study

We found a cachectic wild adult female chimpanzee (*Pan troglodytes verus*) with severe yaws-like lesions on the mouth and lips in a mining concession in Sangaredi area, Guinea (Figure 1A). The chimpanzee was in visible agony, and had to be euthanized; a necropsy was then performed on the body. Gross pathology of the skin revealed a marked depigmentation on hypertrophied edematous lips; crusts and ulcers were present on the head and much of the nose was missing. The eyes were shrunken and purulent and surrounded by crusts and the cornea was opaque. Samples of lesioned skin were preserved in 10% formalin and RNAlater.

Formalin-fixed skin samples were analyzed with both histological and immuno-histochemical methods as previously described (*6*). Histopathological features of the skin biopsies were compatible with treponemal infection (Figure 2A). Skin lesions were characterized by irregular epidermal proliferation of different extents. The epidermis developed hyperkeratosis and hypertrophy of the epidermal rete pegs, which branched and projected deeply into the corium. Admixed areas with severe superficial erosions or deep ulcerations were observed. A moderate to severe mixed cell infiltration composed of lymphocytes and histiocytes was present in the underlying dermal layer. The cellular reaction was most pronounced around the dermal blood vessels and hair follicles, resulting in superficial and deep perivascular dermatitis. The epidermal surface was covered with a dried serosanguineous discharge. Immunohistochemical analyses failed to visualize treponemes, which is a frequent problem due to low numbers of bacteria at lesion sites (*6*).

DNA was extracted from two facial lesion biopsies stored in RNAlater and molecular investigations were performed (supplementary methods). High throughput sequencing analysis resulted in a 24-fold average coverage of the TPE genome, with 98.6% of the genome being covered by at least one read and 97.6% by at least three reads. Bayesian Markov chain Monte Carlo analysis of a genomic alignment comprising this reconstructed TPE genome, all other available TPE and *Treponema pallidum* subsp. *endemicum* (TEN, bejel) genomes and a selection of *Treponema pallidum* subsp. *pallidum* (TPA, syphilis) genomes available in Genbank (Supplementary Table S1) revealed that the chimpanzee derived genome clustered within the well-supported TPE clade, indicating that TPE is responsible for the clinical picture observed in this particular wild chimpanzee (Figure 2B). Interestingly, this new chimpanzee derived genome more precisely belonged to a clade consisting of TPE strains isolated from NHPs in the far West Africa in Gambia, Guinea Bissau, Senegal, and Guinea, in agreement with recent observations that genomic diversity of TPE strains infecting NHPs appears to be geographically structured (*8,10*). Yaws is principally a skin disease and it seems likely that the poor condition of this animal was due to another unknown, likely traumatic cause, perhaps coupled with associated secondary infections, though our field necropsy was not able to identify an alternative cause of her cachectic condition.

To determine whether TPE might affect other chimpanzees in Guinea, we examined videos collected by camera traps set near the Chimpanzee Conservation Center, in Niger National Park. Between 2018 and 2019, 12 individuals (one juvenile, three sub-adults, and eight adults) in 10 different camera trap locations were observed with severe lesions. The lesions observed in these images closely resembled those of the wild female from the Sangaredi region described above, including shrunken eyes, deformation of the face, absence of the nose, and hypertrophied and depigmented lips (and in one case, lips were even completely missing; Figure 1B and 1C).

Molecular investigations of the pathogen(s) causing these infections is clearly warranted, perhaps through non-invasive screening of TPE in feces, bones, or primate associated flies (*10,13*).

Conclusions

This study links yaws-like pathology to the actual detection of TPE in a wild chimpanzee, providing evidence that at least part of the suggestive lesions often observed in wild great apes are caused by this pathogen. These data join a growing body of evidence demonstrating that many non-human primate species across sub-Saharan Africa are infected with TPE (*1,10*). This could potentially be problematic for the ongoing campaign to eradicate TPE globally by 2030 (*14*), though clearly, data from TPE-infected humans in this region are needed to determine whether zoonotic transmission of this pathogen occurs. Given the severity of lesions, it is evident that individual animal fitness is affected. The impact of this disease on NHP populations is unknown but could be assessed through long-term monitoring.

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Data availability

We archived all raw sequence read files in NCBI under BioProject PRJNA588802.

Biography

Benjamin Mubemba is a PhD Student with the research group - Epidemiology of Highly Pathogenic Organisms at the Robert Koch-Institut, Berlin, Germany and Emeline Chanove is the veterinarian in-charge at the Chimpanzee Conservation Center-CCC, Somoria, Faranah, Republic of Guinea. Both Benjamin Mubemba and Emeline Chanove are veterinarians interested in infectious diseases of wildlife with a focus on wild non-human primates.

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Figure 1: A) Yaws-like lesions observed during a necropsy of an adult female chimpanzee in the Sangaredi area, Guinea. Camera trap images showing adult **(B)** and juvenile **(C)** chimpanzees in Niger National Park, Guinea suggesting yaws-like lesions are widespread across chimpanzees in the region.

Figure 2: A) Histopathological evidence suggestive of a treponemal infection. Shown here is superficial ulcerative pyogranulomatous dermatitis including formation of a mixed inflammatory cell infiltration, predominantly neutrophil granulocytes. Deeper dermal layers show the formation of a perivascular lymphocytic inflammatory cell infiltrate, focal folliculitis, and perifolliculitis. Skin areas adjacent to ulcerated parts show irregular epidermal hyperplasia, consistent with treponemal infections. The ulcerated areas were covered by a serocellular crust. **B**) Maximum clade credibility tree of *Treponema pallidum* strain genomes. All simian infecting strains are shown in bold with tip labels showing the species of NHPs. The chimpanzee genome generated

in this study is shown in red. Branches supported by posterior probabilities < 0.95 in the Bayesian Markov chain Monte Carlo tree are indicated in gray. The scale shows nucleotide substitutions per variable site.

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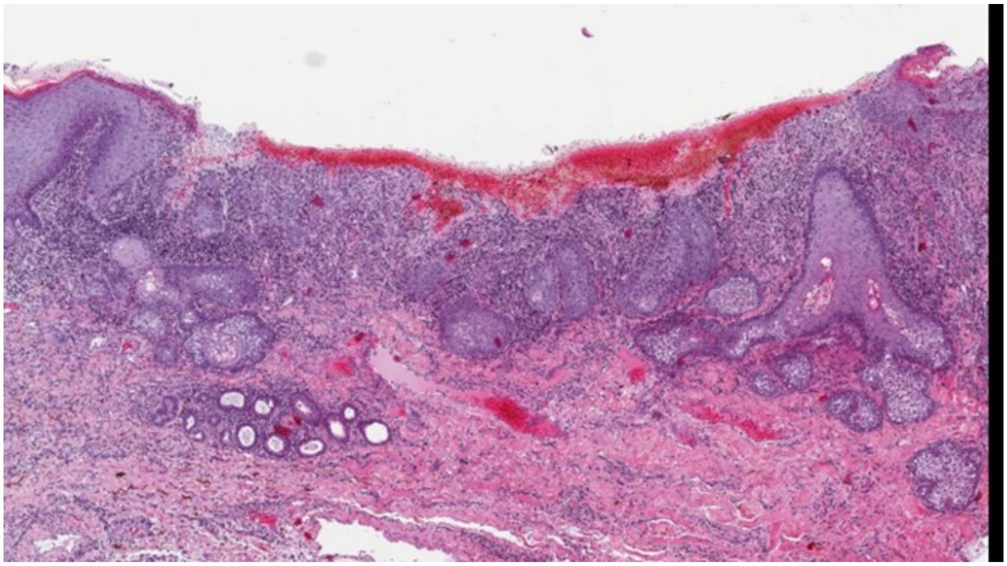

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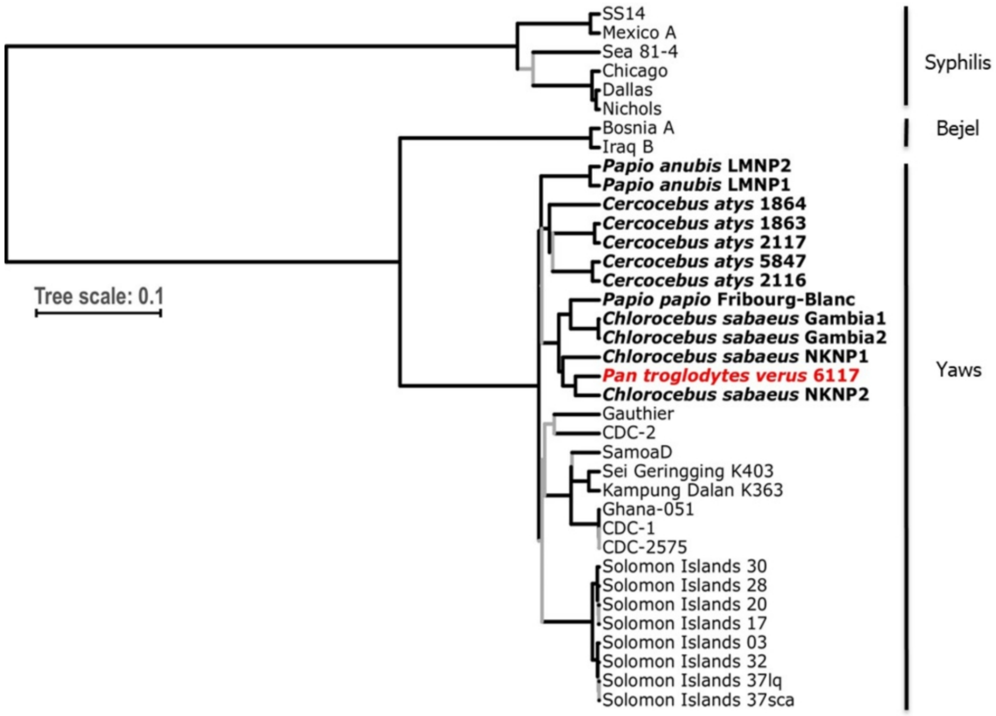
SS14
Mexico A
Sea 81-4
Chicago
Dallas
Nichols
Bosnia A
Iraq B
Papio anubis LMNP2
Papio anubis LMNP1
Cercocebus atys 1864
Cercocebus atys 1863
Cercocebus atys 2117
Cercocebus atys 5847
Cercocebus atys 2116
Papio papio Fribourg-Blanc
Chlorocebus sabaeus Gambia1
Chlorocebus sabaeus Gambia2
Chlorocebus sabaeus NKNP1
Pan troglodytes verus 6117
Chlorocebus sabaeus NKNP2
Gauthier
CDC-2
SamoaD
Sei Geringging K403
Kampung Dalan K363
Ghana-051
CDC-1
CDC-2575
Solomon Islands 30
Solomon Islands 28
Solomon Islands 20
Solomon Islands 17
Solomon Islands 03
Solomon Islands 32
Solomon Islands 37lq
Solomon Islands 37sca
Syphilis
Bejel
Yaws
Tree scale: 0.1

Supplementary methods

Molecular and bioinformatics analyses

DNA was extracted from two facial lesion biopsies using the DNeasy blood and tissue extraction kit (Qiagen, Germany) following the manufacturer's protocol. Samples were screened with a qPCR targeting the *pol*A gene of Treponema *pallidum* as described in (*1*) and both samples were positive. DNA extracts were converted into dual indexed Illumina libraries using the NEBNext® Ultra™ II DNA Library Prep kit (New England Biolabs®). Libraries were enriched for TPE through in-solution hybridization capture as previously described in (*2,3*) and sequenced on an Illumina NextSeq (v2 chemistry, 2x150-cycles). Reads were quality filtered using Trimmomatic v0.38 (removing leading and trailing reads <Q30; clipping reads where average base quality across 4 bp was <30; removing surviving reads <30 nt long) (*4*). Surviving read pairs were merged with Clip and Merge v1.7.8. Merged reads and surviving singletons were combined and mapped to TPE Fribourg-Blanc (RefSeq ID: NC_021179.1) using BWA-MEM with a minimum seed length of 29. Mapped reads were sorted using Picard's SortSam, de-duplicated with Picard's MarkDuplicates (https://broadinstitute.github.io/picard/index.html), and alignments with MAPQ <30 and a mapping length <30 nt were removed using SAMtools (*5*). Finally, we merged all mapped reads of individual library samples to produce single TPE draft genome. We used Geneious v11 to call a consensus genome requiring a minimum of 3 unique reads to cover a position for it to be called and applying a majority consensus rule (*6*).

Whole genome alignment was performed using the multiple sequence alignment program MAFFT (*7*). We then removed all putative recombinant genes (*3*) and selected conserved blocks using the Gblocks tool (*8*) in SeaView v4 (*9*). The Bayesian Markov Chain Monte Carlo phylogenomic analysis was performed in BEAST (version 1.10.4) on the resulting alignment of

4213 variable positions (after stripping off of all ambiguities and identical sites in the final data set); settings of the analysis were a strict clock model and a coalescent process assuming constant population size. The output of multiple chains of 10,000,000 generations was examined for convergence and appropriate sampling of the posterior using Tracer (version 1.7.1) (*10*) before merging tree files using Log Combiner (version 1.10.4) (*11*). The best representative tree was picked from the posterior set of trees and annotated with Tree Annotator (version 1.10.4: distributed with BEAST). The resultant maximum clade credibility (MCC) tree file was further edited using iTOL (https://itol.embl.de/) (*12*).

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Supplementary Table S1: Published *Treponema pallidum* sequences used in this study

Isolate ID	GenBank Accession ID	Host	TP spectrum	Country	Reference
Bosnia A	CP007548.1	*Homo Sapiens*	Bejel	Bosnia	1
Iraq_B	CP032303.1	*Homo Sapiens*	Bejel	Iraq	2
Nichols	NC_021490.2	*Homo Sapiens*	Syphilis	USA	3
SS14	NC_021508.1	*Homo Sapiens*	Syphilis	USA	3
Chicago	NC_017268.1	*Homo Sapiens*	Syphilis	USA	4
Mexico A	NC_018722.1	*Homo Sapiens*	Syphilis	Mexico	3
Dallas	NC_016844.1	*Homo Sapiens*	Syphilis	USA	5
Seattle 81-4	CP003679.1	*Homo Sapiens*	Syphilis	USA	6
Fribourg-Blanc	NC_021179.1	*Papio papio*	Yaws	Guinea	7
Samoa D	NC_016842.1	*Homo Sapiens*	Yaws	Samoa	8
Gauthier	NC_016843.1	*Homo Sapiens*	Yaws	Republic of the Congo	8
CDC-1	CP024750.1	*Homo Sapiens*	Yaws	Ghana	9
CDC-2	NC_016848.1	*Homo Sapiens*	Yaws	Ghana	8
CDC_2575	CP020366	*Homo Sapiens*	Yaws	Ghana	10
Ghana-051	CP020365	*Homo Sapiens*	Yaws	Ghana	10
Kampung_Dalan_K363	CP024088.1	*Homo Sapiens*	Yaws	Indonesia	11
Sei_Geringging_K403	CP024089.1	*Homo Sapiens*	Yaws	Indonesia	11
Solomon Islands 03	ERR1470343	*Homo Sapiens*	Yaws	Solomon Islands	12
Solomon Islands 17	ERR1470344	*Homo Sapiens*	Yaws	Solomon Islands	12
Solomon Islands 20	ERR1470335	*Homo Sapiens*	Yaws	Solomon Islands	12
Solomon Islands 28	ERR1470338	*Homo Sapiens*	Yaws	Solomon Islands	12
Solomon Islands 30	ERR1470334	*Homo Sapiens*	Yaws	Solomon Islands	12
Solomon Islands 32	ERR1470342	*Homo Sapiens*	Yaws	Solomon Islands	12
Solomon Islands 37 liq	ERR1470330	*Homo Sapiens*	Yaws	Solomon Islands	12
Solomon Islands 37 sca	ERR1470331	*Homo Sapiens*	Yaws	Solomon Islands	12

Gambia-1	SRR4308597	*Chlorocebus sabeus*	Yaws	Gambia	13
Gambia-2	SRR4308605	*Chlorocebus sabaeus*	Yaws	Gambia	13
Senegal NKNP-1	SRR4308606	*Chlorocebus sabaeus*	Yaws	Senegal	13
Senegal NKNP-2	SRR4308607	*Chlorocebus sabaeus*	Yaws	Senegal	13
LMNP-1	CP021113.1	*Papio anubis*	Yaws	Tanzania	13
LMNP-2_BS5	SRR4308598	*Papio anubis*	Yaws	Tanzania	13
LMNP-2_BS6	SRR4308599	*Papio anubis*	Yaws	Tanzania	13
LMNP-2_BS7	SRR4308601	*Papio anubis*	Yaws	Tanzania	13
LMNP-2_BS8	SRR4308602	*Papio anubis*	Yaws	Tanzania	13
1863-Hato	SRR4308604	*Cercocebus atys*	Yaws	Côte d'Ivoire	13
1864-IGU	SRR4308596	*Cercocebus atys*	Yaws	Côte d'Ivoire	13
2117-BAK	SAMN13258074	*Cercocebus atys*	Yaws	Côte d'Ivoire	14
2116-OKA	SAMN13258075	*Cercocebus atys*	Yaws	Côte d'Ivoire	14
5847-CHAT	SAMN13258076	*Cercocebus atys*	Yaws	Côte d'Ivoire	14

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