

Molecular Detection and Characterization of *Rickettsia asembonensis* in Human Blood, Zambia

Lavel C. Moonga, Kyoko Hayashida, Namwiinga R. Mulunda, Yukiko Nakamura, James Chipeta,¹ Hawela B. Moonga, Boniface Namangala, Chihiro Sugimoto, Zephaniah Mtonga, Mable Mutengo, Junya Yamagishi

Author affiliations: Hokkaido University, Sapporo, Japan (L.C. Moonga, K. Hayashida, Y. Nakamura, C. Sugimoto, J. Yamagishi); University of Zambia, Lusaka, Zambia (N.R. Mulunda, J. Chipeta, B. Namangala, M. Mutengo); National Malaria Control Center, Lusaka (H.B. Moonga); Chongwe District Hospital, Lusaka (Z. Mtonga); Levy Mwanawasa Medical University, Lusaka (M. Mutengo)

DOI: <https://doi.org/10.3201/eid2708.203467>

Rickettsia asembonensis is a flea-related *Rickettsia* with unknown pathogenicity to humans. We detected *R. asembonensis* DNA in 2 of 1,153 human blood samples in Zambia. Our findings suggest the possibility of *R. asembonensis* infection in humans despite its unknown pathogenicity.

Rickettsia asembonensis is a fleaborne rickettsia closely related to *Rickettsia felis* and is thus referred to as an *R. felis*-like organism. *R. asembonensis* was first detected in cat fleas in Kenya and subsequently reported worldwide (1,2). Although *R. felis* has been increasingly recognized as a human infective agent that can cause human febrile disease, the infectivity and pathogenicity of *R. asembonensis* in humans is largely unknown. Recent investigations in patients with febrile illness and petechial lesions identified *R. asembonensis* DNA and antibodies for rickettsial antigens in Malaysia (3,4). Furthermore, *R. asembonensis* was isolated in cellular cultures from patients in Peru with acute febrile illness and confirmed by sequencing (5). These reports suggest the possibility of *R. asembonensis* as a human infective agent. However, no direct evidence of *R. felis* and *R. asembonensis* as an etiologic agent of human illness has been established. A previous study in Zambia revealed the predominant existence of *R. asembonensis* and *R. felis* in cat fleas (6). Our study investigates the presence of these rickettsiae in human blood in Zambia.

We obtained 753 residual patient blood samples from hospitals in urban Lusaka (n = 519) and the

Chongwe District (n = 234) of Zambia. Approximately half of the samples (303/753) were traceable to clinical records of patients. The common clinical conditions among those patients included fever, anemia, meningitis, septicemia, and sickle cell anemia (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/27/8/20-3467-App1.pdf>). In addition, we obtained dried blood spots on Whatman FTA classic cards (Millipore Sigma, <https://www.sigmaaldrich.com>) from healthy volunteers from rural eastern (n = 200) and central (n = 200) provinces to assess rickettsia infection in healthy rural persons. The study was approved by the National Health Research Authority of Zambia through the Biomedical Research Ethics Committee (reference no. 007-10-18).

We extracted genomic DNA and subjected it to PCR screening that targeted the citrate synthase gene (*gltA*) of *Rickettsia*. We subjected the positive samples to multiple-gene sequencing analysis targeting the 17-kDa common antigen (*htrA*), outer membrane protein A (*OmpA*), and outer membrane protein B (*OmpB*) genes using previously described primers (Appendix Table 2). We aligned the sequences using MAFFT (<https://mafft.cbrc.jp/alignment/server>) and performed phylogenetic analysis by the neighbor-joining method using MEGA7 (<https://www.megasoftware.net>). We determined the estimated *Rickettsia* bacterial burden in *Rickettsia*-positive blood samples by *OmpA* quantitative PCR by using published primers. We further tested the *gltA* PCR-positive samples for malaria by nested PCR (Appendix Table 2).

We detected *R. asembonensis* in 0.39% (2/519) samples from the urban Lusaka District by *gltA* PCR. The samples from the Chongwe District and the rural areas of the eastern and central provinces were all negative, although the possibility that dried blood spot samples from rural areas might have lower detection sensitivity cannot be ruled out. BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) of the *gltA* sequences obtained (GenBank accession nos. LC557154 and LC557155) showed 100% homology to *R. asembonensis* identified in cat fleas from human dwellings and domestic dogs in 3 countries: Senegal (GenBank accession no. JF966774), Kenya (accession no. JN315968), and Zambia (accession no. LC431490) (6,7). Comparing the sequenced *gltA* with those detected in fleas from Peru (GenBank accession no. KY650697) and other regions in the Americas showed 99.8% similarity. Phylogenetic analysis of *gltA* confirmed the detected sequences' closer relatedness by clustering with genes from cat fleas from sub-Saharan Africa, a distinct cluster from other regions (Figure). The *OmpA*, *OmpB*, and *htrA* sequences showed clustering without regional

¹Deceased.

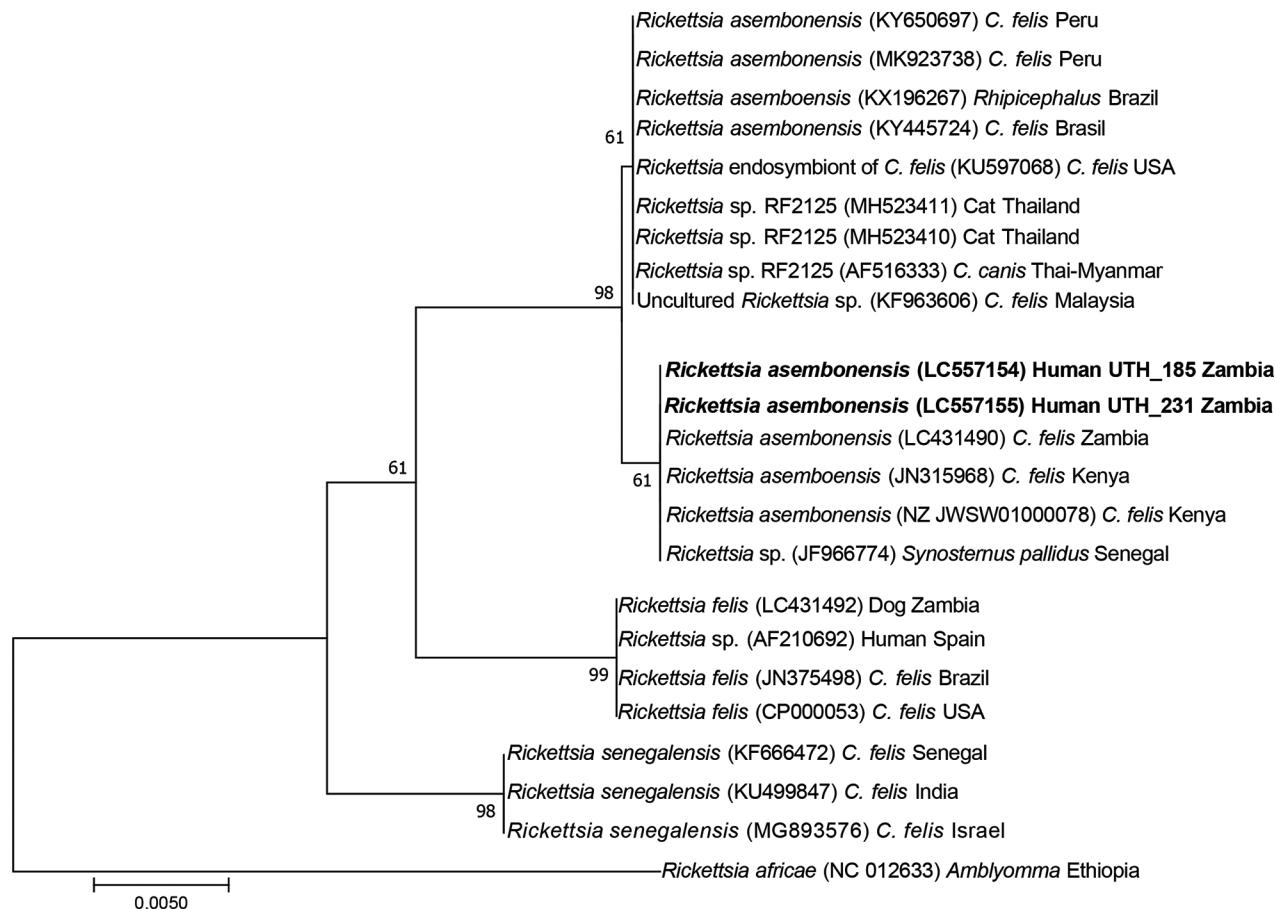


Figure. Phylogenetic tree of *Rickettsia felis* and *R. felis*-like organisms based on the sequences of the *gltA* gene (581 bp) from human blood samples collected from Zambia, 2019 (in bold). The tree was constructed using the neighbor-joining method with the maximum-likelihood model. Bootstrap values are shown on nodes based on 1,000 replicates. Sequences are identified by species name, GenBank accession number, host, and country of detection. Scale bar indicates nucleotide substitutions per site.

discrimination (data not shown). The obtained nucleotides are available in GenBank under accession nos. LC557154–61. Detection of genotypically similar *R. asemonensis* in persons and cat fleas in Zambia suggests possible human infection by *R. asemonensis* through cat flea bites. Nevertheless, the epidemiologic cycle and pathogenicity of *R. asemonensis* and other related *R. felis*-like organisms remain to be elucidated.

The patient identified as UTH_185 in whom *R. asemonensis* was detected had a medical record of anemia and weight loss (Table). The malaria test was

negative. Despite the limited association of *Rickettsia* infection with anemia, severe *R. felis* infection has been reported with severe anemia, possibly attributable to hemorrhage from vascular damage in rickettsial disease (8). However, the observed evidence was limited and could not establish *R. asemonensis* as the cause of these symptoms. The second *R. asemonensis*-positive sample from the patient identified as UTH_231 had limited clinical information, which did not allow for further interpretation. The 2 *R. asemonensis*-positive blood samples showed estimated

Table. Selected demographic and clinical characteristics of 2 persons in whom *Rickettsia asemonensis* was detected from blood samples collected in Zambia

Characteristic	Patient UTH_185	Patient UTH_231
Age, y	42	45
Sex	Female	Female
Residential area	Lusaka	Lusaka
Clinical manifestation	Anemia and weight loss	No information
Estimated rickettsia genome copies/mL blood	890,000	2,150,000
Malaria test	Negative	Negative

DNA quantities of 890,000 copies/mL of blood from patient UTH_185 and 2,100,000 copies/mL of blood from patient UTH_231 (Table). These results are within the same range as a previous study for *Rickettsia rickettsii* estimated rickettsial burden (9).

In conclusion, detection of *R. asembonensis* of identical genotype in cat fleas and human blood in Zambia suggests possible transmission from cat fleas to humans. Given the worldwide distribution of *R. asembonensis*, further studies to elucidate its pathogenicity and epidemiologic cycle are warranted.

This study was supported by the Japan Agency for Medical Research and Development (grant no. JP21wm0125008). The funder had no role in the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript. This work is dedicated to the memory of James Chipeta. May his soul rest in peace.

About the Author

Dr. Moonga is a postdoctoral research fellow at Hokkaido University. His research interests include arthropodborne zoonoses, specifically fleaborne rickettsia infections as a possible cause of febrile illness, and developing of rapid molecular diagnostic tools.

References

- Jiang J, Maina AN, Knobel DL, Cleaveland S, Laudisoit A, Wamburu K, et al. Molecular detection of *Rickettsia felis* and *Candidatus Rickettsia asemboensis* in fleas from human habitats, Asembo, Kenya. *Vector Borne Zoonotic Dis.* 2013;13:550–8. <https://doi.org/10.1089/vbz.2012.1123>
- Maina AN, Jiang J, Luce-Fedrow A, St John HK, Farris CM, Richards AL. Worldwide presence and features of flea-borne *Rickettsia asembonensis*. *Front Vet Sci.* 2019;5:334. <https://doi.org/10.3389/fvets.2018.00334>
- Kho KL, Koh FX, Singh HKL, Zan HAM, Kukreja A, Ponnampalavanar S, et al. Case report: spotted fever group rickettsioses and murine typhus in a Malaysian teaching hospital. *Am J Trop Med Hyg.* 2016;95:765–8. <https://doi.org/10.4269/ajtmh.16-0199>
- Tay ST, Kho KL, Vythilingam I, Ooi CH, Lau YL. Investigation of possible rickettsial infection in patients with malaria. *Trop Biomed.* 2019;36:257–62.
- Palacios-Salvatierra R, Cáceres-Rey O, Vásquez-Domínguez A, Mosquera-Visaloth P, Anaya-Ramírez E. Rickettsial species in human cases with non-specific acute febrile syndrome in Peru [in Spanish]. *Rev Peru Med Exp Salud Publica.* 2018;35:630–5. <https://doi.org/10.17843/rpmesp.2018.354.3646>
- Moonga LC, Hayashida K, Nakao R, Lisulo M, Kaneko C, Nakamura I, et al. Molecular detection of *Rickettsia felis* in dogs, rodents and cat fleas in Zambia. *Parasit Vectors.* 2019;12:168. <https://doi.org/10.1186/s13071-019-3435-6>
- Roucher C, Mediannikov O, Diatta G, Trape J-FF, Raoult D. A new *Rickettsia* species found in fleas collected from human dwellings and from domestic cats and dogs in Senegal. *Vector Borne Zoonotic Dis.* 2012;12:360–5. <https://doi.org/10.1089/vbz.2011.0734>
- Zavala-Castro J, Zavala-Velázquez J, Walker D, Pérez-Osorio J, Peniche-Lara G. Severe human infection with *Rickettsia felis* associated with hepatitis in Yucatan, Mexico. *Int J Med Microbiol.* 2009;299:529–33. <https://doi.org/10.1016/j.ijmm.2009.03.002>
- Kato C, Chung I, Paddock C. Estimation of *Rickettsia rickettsii* copy number in the blood of patients with Rocky Mountain spotted fever suggests cyclic diurnal trends in bacteraemia. *Clin Microbiol Infect.* 2016;22:394–6. <https://doi.org/10.1016/j.cmi.2015.12.019>

Address for correspondence: Kyoko Hayashida, Hokkaido University, International Institute for Zoonosis Control, Division of Collaboration and Education, Kita 20 Nishi 10, Kita-Ku, Sapporo 001-0020, Hokkaido, Japan; email: kyouko-h@czc.hokudai.ac.jp

Molecular Detection and Characterization of *Rickettsia asembonensis* Human Blood, Zambia

Appendix

Appendix Table 1. Classification of clinical manifestation of sampled individuals obtained from hospitals in Lusaka and Chongwe, Zambia.

Clinical manifestation or disease name	Number of samples in the category
Febrile illness	10
Neurologic symptoms (meningitis)	12
Septicemia	22
Anemia	36
Sickle cell anemia	45
HIV	18
Malaria	24
Respiratory infection	49
Others	87
No clinical information provided	450
Total	753

Appendix Table 2. Primers for amplification of genes from *Rickettsia* spp. and *Plasmodium* spp

Target gene	Target pathogen	Annealing temperature (°C)	Amplicon size (bp)	Oligonucleotide sequence (5'-3')	Reference
Citrate synthase gene (<i>gltA</i>)	<i>Rickettsia</i> genus	54	581	Forward: <i>gltA_Fc</i> CGAACTTACCGCTATTAGAATG	(1)
				Reverse: <i>gltA_Rc</i> CTTTAAGAGCGATAGCTTCAAG	
Outer membrane A gene (<i>ompA</i>)*	Spotted fever group rickettsia	48	258	Forward: <i>ompA_Fm</i> TGCAGGGCTTAGATATTCGGC	(2)
				Reverse: <i>ompA_Rm</i> AAGCTGTTGGTAAAGGAGCA	
Outer membrane protein B gene (<i>ompB</i>)	Spotted fever group rickettsia	48	776	Forward: <i>ompB_Fm</i> GGACCTGAAGCTGGAGCAAT	(2)
				Reverse: <i>ompB_Rm</i> CTGTCAGGCTGGCTGATGAA	
17kDa common antigen gene (<i>htrA</i>)	<i>Rickettsia</i> genus	50	545	Forward: 17k_5 GCTTTACAAAATTCTAAAAACCATATA	(3)
				Reverse: 17k_3 TGTCTATCAATTCACAACCTTGCC	
Small subunit ribosomal RNA gene (SSU rRNA)	<i>Plasmodium</i> genus	60	620	Forward: rPLU 1 TCAAAGATTAAGCCATGCAAGTGA	(4)
				Reverse: rPLU 5 CCTGTTGTTGCCTTAAACTTC	
Small subunit ribosomal RNA genes (SSU rRNA)	<i>Plasmodium</i> genus (nested-PCR)	60	240	Forward rPLU 3: TTTTTATAAGGATAACTACGAAAAGCTGT	(4)
				Forward rPLU 4: TACCCGTCATAGCCATGTTAGGCCAATACC	

* *OmpA* primer set used for estimation of rickettsial load by quantitative real-time PCR using SYBR Green assay.

References

1. Gaowa, Ohashi N, Aochi M, Wuritu D, Wu DX, Yoshikawa Y, et al. *Rickettsiae* in ticks, Japan, 2007–2011. *Emerg Infect Dis*. 2013;19:338–40. [PubMed https://doi.org/10.3201/eid1902.120856](https://doi.org/10.3201/eid1902.120856)
2. Moonga LC, Hayashida K, Nakao R, Lisulo M, Kaneko C, Nakamura I, et al. Molecular detection of *Rickettsia felis* in dogs, rodents and cat fleas in Zambia. *Parasit Vectors*. 2019;12:168. [PubMed https://doi.org/10.1186/s13071-019-3435-6](https://doi.org/10.1186/s13071-019-3435-6)
3. Labruna MB, Whitworth T, Bouyer DH, McBride J, Camargo LM, Camargo EP, et al. *Rickettsia bellii* and *Rickettsia amblyommii* in *Amblyomma* ticks from the state of Rondônia, Western Amazon, Brazil. *J Med Entomol*. 2004;41:1073–81. [PubMed https://doi.org/10.1603/0022-2585-41.6.1073](https://doi.org/10.1603/0022-2585-41.6.1073)
4. Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MS, Rahman HA. A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. *Am J Trop Med Hyg*. 1999;60:687–92. [PubMed https://doi.org/10.4269/ajtmh.1999.60.687](https://doi.org/10.4269/ajtmh.1999.60.687)