highlights the need to further investigate the infectivity and pathophysiology of the virus located in the often neglected capillary compartment. These findings provide new information on this biologic compartment, which plays a key role in vectorborne transmission and transmission dynamics. Moreover, these observations, if validated with more patients and extended to other vectorborne infections, will be vital for preventing and controlling the transmission of Zika virus and other arboviruses.

Institutional review board approval was granted by the Comité de Protection des Personnes Sud-Méditerranée I corresponding to the following study “Etude descriptive prospective de la maladie à virus Zika au sein de la communauté de défense des Forces Armées en Guyane” and was registered February 2016 under the number RCB: 2016-A00394-47. Written informed consent was obtained from each patient as required by the Comité de Protection des Personnes Sud-Méditerranée I.

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References


Detection of Spotted Fever Group Rickettsia DNA by Deep Sequencing

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After conventional molecular and serologic testing failed to diagnose the cause of illness, deep sequencing identified spotted fever group Rickettsia DNA in a patient’s blood sample. Sequences belonged to R. honei, the causative agent of Flinders Island spotted fever. Next-generation sequencing is proving to be a useful tool for clinical diagnostics.

When conventional laboratory tests cannot identify an etiologic agent, unbiased deep sequencing performed directly on a clinical sample has the potential to identify a probable cause of disease. We used deep sequencing to detect spotted fever group (SFG) Rickettsia DNA in the blood of a patient for whom diagnosis was not possible through conventional molecular and serologic testing.

In late 2016, a middle-aged woman was admitted to a regional hospital in Queensland, Australia, after 2 weeks of mild cough, myalgia, fever, and lethargy. The day before admission, she experienced a blanching rash and pains in her feet, after which her condition deteriorated and a definite petechial rash appeared. Chest radiographs showed atelectasis on 1 side. Meningococcal septicemia was suspected, and the patient was transferred to intensive care with septic shock. Despite treatment with inotropes and several antimicrobial drugs (including ceftriaxone, vancomycin, meropenem, doxycycline), the patient died the next morning.

Clinical testing did not identify an infectious disease agent in the patient’s blood; serologic test results

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for *Rickettsia* were negative. Because a limited amount of specimen remained for testing, we applied an unbiased deep-sequencing approach. We extracted DNA from the blood sample by using the MasterPure Complete DNA Purification Kit (Epicenter, Madison, WI, USA) and sequenced with the Ion Torrent PGM (Personal Genome Machine) workflow by using the Ion PGM IC 200 Kit and the Ion 316 Chip Kit, version 2 (Life Technologies, Carlsbad, CA, USA). A total of 3,627,903 sequences were generated and trimmed by using a minimum quality score of Q15 and minimum length of 50 bp. Of the reads generated, 251 matched bacterial DNA sequences (uploaded to GenBank as Bioproject PRJEB21107). The rest either matched human genome sequences and were filtered out (3,619,386 reads) or were unclassified (8,252 reads).

We analyzed the reads for bacterial DNA by using 3 metagenomics tools: Kraken (1), PathoScope (2), and One Codex (https://www.onecodex.com). All 3 analyses returned similar results; ≈80% of classified reads (208/251 reads, 53,958 total nucleotides) matched sequences from SFG *Rickettsia* spp.; the remainder gave low-number, low-quality matches to other bacteria. Screening of reads for sequences matching 5 rickettsial genes (*rrs, ompA, ompB, gltA*, and *sea4*) found 1 read mapping to the *ompB* gene (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/11/17-0474-Techapp1.pdf). This read was a 100% match (272/272 nt) to *R. honei ompB* (GenBank accession nos. AF123724.1, AF123711.1). The next highest match was to *R. parkeri* *ompB* (accession no. KY113111.1) at 99% (270/272 nt). We confirmed the presence of SFG *Rickettsia* DNA in the DNA extract of the sample by nested PCR and performed Sanger sequencing by using the Invitrogen SuperScript III One-Step RT-PCR system with primers (3) and in-house nested primers.

To narrow down the identification to species level, we further analyzed sequences matching *Rickettsia* spp. We downloaded all *Rickettsia* genomes available at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), complete and draft, and used them as reference sequences for mapping of the reads in CLC Genomics Workbench 8 (QIAGEN Aarhus, Silkeborgvej, Denmark). We discarded reads mapping to >1 genome, collected the remaining reads that mapped uniquely to a single genome, and noted the genome to which they mapped. Of the 208 reads, 67 mapped to >1 genome, 1 did not map to any of the genomes and was subsequently identified as matching that of the human reference genome, 3 were unique matches to *R. conorii* (AJURO1, GenBank accession no. NC_003103), 1 was a unique match to *R. sibirica* (accession no. NZ_AHZB01000018), and 151 were unique matches to *R. honei* (accession no. NZ_AJTT0000000) (online Technical Appendix) (4). Mapping of the 208 sequencing reads revealed that 207 (99.6%) reads mapped to the *R. honei* genome, giving 1.43% coverage of the genome, and 168 (80.7%) reads mapped to the *R. australis* (accession no. NC_017058) genome, representing 0.03% coverage of the genome.

The main causes of SFG rickettsioses in Australia are *R. australis* and *R. honei*, which cause Queensland tick typhus and Flinders Island spotted fever, respectively (5). The rickettsial DNA in the blood sample we describe most closely matched sequences from *R. honei* and had a relatively low level of similarity to sequences from *R. australis*. *R. honei* was initially reported only in the southern states of Australia; however, a genetic variant known as the “marmionii” strain has since been reported in eastern and northern parts of the country (6). Unfortunately, the genome of *R. honei* “marmorii” has not been sequenced, and the genes used to differentiate between *R. honei* and *R. honei* “marmionii” were not covered by the sequences generated from the sample. Therefore, we could not confirm which strain of *R. honei* was in the sample.

Flinders Island spotted fever is reportedly associated with relatively mild illness (5). However, our detection of *R. honei* DNA in the blood of a deceased patient, in the absence of positive *Rickettsia* serologic test results, is suggestive of acute infection with this agent. This case demonstrates the potential of deep sequencing for identifying unknown etiologic agents, particularly when other methods have not done so.

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Dr. Graham is a senior scientist in the Molecular Epidemiology Unit of the Queensland Department of Health Public Health Microbiology Laboratory at Forensic and Scientific Services. Her work involves research into the potential of whole-genome sequencing as a tool for public health microbiology.

**References**

Chlamydia trachomatis Biovar L2 Infection in Women in South Africa

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We detected Chlamydia trachomatis biovar L2 in vaginal swab specimens of 7 women with vaginal discharge in South Africa. Whole-genome sequencing directly from clinical specimens identified a closely related cluster of strains. The clinical role of this infection in the context of syndromic management should be clarified.

Infection with Chlamydia trachomatis biovar L is known as lymphogranuloma venereum (LGV). This infection usually presents as genital ulcers, followed by an invasion of the lymphatic system resulting in buboes, painful swelling of lymph nodes (1). In the past 2 decades, another manifestation of LGV has emerged in North America and Europe: rectal LGV infection causing proctocolitis among men who have sex with men (MSM) (1). In this population, urethral LGV also occurs (2).

There have been only sporadic reports of rectal and genital LGV infection in women living in the industrialized world (3,4). Cross-sectional studies from France, Switzerland, and the Netherlands did not detect biovar L in specimens from women with genital or rectal C. trachomatis infection (1,5–7). Because lymphatic manifestation has become relatively rare, LGV infection is considered an outbreak mainly among MSM in Europe and North America (1). Lymphatic LGV is endemic to Africa, but before our study, it was unknown whether C. trachomatis biovar L infections occurred in women in Africa. Thus, we determined the prevalence of this infection in South Africa.

To determine whether genital C. trachomatis biovar L infections occur in women living in South Africa, we analyzed 82 DNA samples extracted from vaginal swab specimens that were positive by a molecular detection assay for C. trachomatis infection at the Department of Medical Microbiology at the University of Pretoria. The Faculty of Health Sciences Research Ethics Committee at the University of Pretoria approved the studies in which these specimens were collected. These swab specimens had been collected during 2012–2016 from women attending different healthcare settings: a mobile health clinic in rural Mopani District (n = 52) and 3 departments at the academic hospital in Pretoria: obstetrics and gynecology clinic (n = 14), antiretroviral treatment clinic (n = 10), and sexually transmitted infection (STI) clinic (n = 6). We assessed the presence of LGV in these genital specimens by using specific PCRs for C. trachomatis serovar L and serovar L2b (8). For positive PCR results, we confirmed the diagnosis by conducting whole-genome sequencing (WGS) of C. trachomatis directly from the clinical specimen as described elsewhere (9).

Whereas C. trachomatis biovar L–specific PCR showed positive results for 7 specimens obtained from women at the antiretroviral treatment (n = 5) and STI (n = 2) clinics in Pretoria, we did not detect LGV in any of the 52 specimens from women in Mopani District. All PCR test results for serovar L2b were negative. The 7 women with genital LGV all had vaginal discharge and were co-infected with another STI (Table).

WGS confirmed LGV (ompA sequence identical to those of the C. trachomatis L2 434/BU reference strain) in 4 cases with good mean read depth (≥12) and high genome coverage (>98%). The 4 sequences clustered well with the L2 sequences previously published and away from L1 and L2b sequences. For 1 specimen, the mean read depth


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