

Modern Solutions for Ancient Pathogens: Direct Pathogen Sequencing for Diagnosis of Lepromatous Leprosy and Cerebral Coenurosis

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Microbes unculturable in vitro remain diagnostically challenging, dependent historically on clinical findings, histology, or targeted molecular detection. We applied whole-genome sequencing directly from tissue to diagnose infections with mycobacteria (leprosy) and parasites (coenurosis). Direct pathogen DNA sequencing provides flexible solutions to diagnosis of difficult pathogens in diverse contexts.

Keywords. leprosy; coenurosis; *Taenia*; mycobacteria; whole genome sequencing.

More than 200 000 new cases of leprosy occur globally annually [1]. Microbiological diagnosis of *Mycobacterium leprae* remains difficult. In vitro culture is unavailable in routine clinical practice. Diagnosis is therefore usually clinical in high-incidence areas and may be delayed, particularly in low-prevalence settings where diagnostic expertise is centralized [2, 3].

Human coenurosis is caused by ingestion of certain *Taenia* tapeworm eggs, which produces parasitic tissue cysts when larval forms breach the intestinal wall. Clinical and radiological features are similar to neurocysticercosis caused by *Taenia solium* [4].

We describe the application of whole-genome sequencing (WGS) direct from tissue to diagnose both leprosy and coenurosis, informing epidemiology and treatment.

Clinical Case 1

A 48-year-old woman presented with painful eyes and leg ulcers. She described burning pain in both lower legs over 6 months, developing tender red swellings that ulcerated and bled, and 5 months of painful, watering eyes, with blurred vision in the left eye. She had lost 7 kg in weight and complained of nasal congestion, with no other symptoms on systemic enquiry. She reported previous treatment for burning leg pain but was unsure of the medical details. She had otherwise been well previously. She was born in a Maritime Southeast Asian nation and had lived in the United Kingdom for 6 years, working as a cleaner. She was a non-smoker who drank no alcohol. She lived with her husband and adult children, all of whom were well.

Clinical examination revealed mild splenomegaly and bilateral inguinal lymphadenopathy. She had multiple deep, irregular ulcers over both legs with red bases and sloughy edges (Figure 1), a fine papular rash over her cheeks and nose, and annular lesions her left knee and thigh. Neurological examination was normal.

Computed tomography of the chest, abdomen, and pelvis confirmed splenomegaly (16 cm) and bilateral non-necrotic inguinal lymphadenopathy (3 cm). Biopsy of a leg ulcer edge was culture negative for bacteria, mycobacteria, and fungi, but histological examination of skin and inguinal lymph node biopsies showed non-necrotizing granulomas and abundant mycobacteria. Molecular testing for *Mycobacterium tuberculosis* was negative.

Lepromatous leprosy and type 2 reaction (erythema nodosum leprosum) were suspected. Ophthalmological examination revealed a left corneal ulcer with hypoesthesia and bilateral sub-epithelial punctate keratitis (Figure 1), multiple small white iris deposits, and intermediate uveitis—all consistent with multibacillary *Mycobacterium leprae* infection. The patient's family then reported that 18 years earlier she had received 12 months of multidrug therapy for presumed leprosy (but had not herself been informed of this diagnosis). *Mycobacterium leprae* infection was confirmed by WGS of lymph node tissue 13 days after biopsy. Treatment was started with rifampicin, ofloxacin, and minocycline.

Clinical Case 2

A 54-year-old woman presented to the hospital in Ghana with right-sided sensory seizures. She had no previous medical history. Magnetic resonance imaging (MRI) of the brain showed a single, left parietal, rim enhancing lesion with significant

Received 01 June 2022; editorial decision 15 August 2022; accepted 21 August 2022; published online 23 August 2022

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Open Forum Infectious Diseases®

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<https://doi.org/10.1093/ofid/ofac428>

surrounding edema ([Supplementary Figure 1](#)). She was started on anti-epileptic medication (levetiracetam) and corticosteroids and referred to a UK specialist neurosurgical unit for biopsy of a suspected high-grade malignancy. Repeat MRI 3 months later showed significant improvement in edema and reduction in the size of a cystic enhancing lesion. Examination was normal apart from mildly reduced power in all muscle groups of the right arm and leg. She underwent navigation-guided left parietal mini-craniotomy and resection.

At surgery, the cortical surface overlying the lesion had a yellowish tinge. The lesion had a thick, firm capsule, allowing complete resection. Microbiological examination of tissue showed no organisms on gram stain, and culture for bacteria, mycobacteria, and fungi was negative.

Histological examination of tissue showed multiple scoleces within a partially degenerate cyst ([Figure 2](#)), without laminated structure to the cyst wall. There were no neoplastic features, but there was reactive brain parenchyma, with perivascular

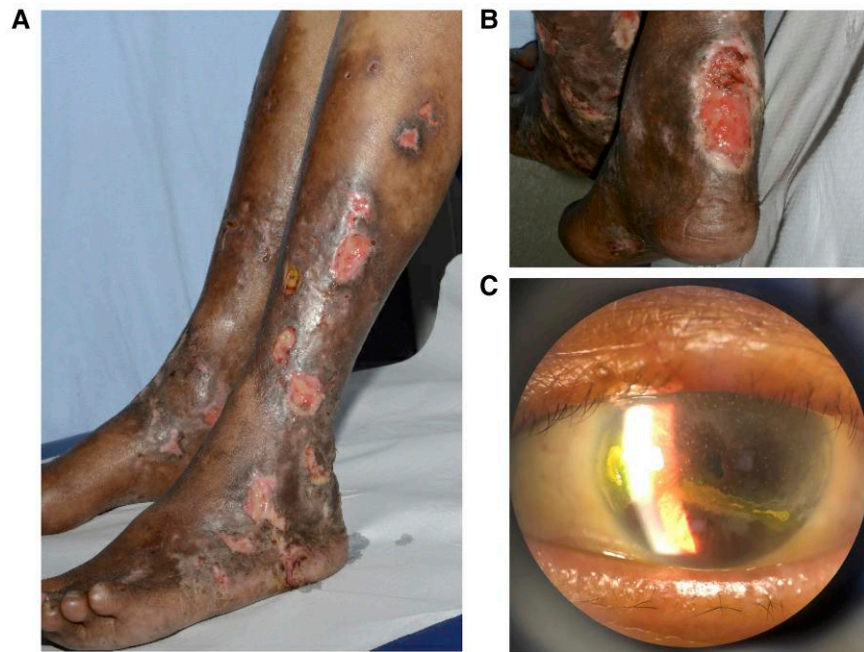


Figure 1. Clinical images from case 1: multibacillary leprosy with type 1 reaction (erythema nodosum leprosum). The patient presented with multiple irregular deep ulcers over both lower limbs (A, B). C, Examination of the left eye showing thickened eyelid, eyelash loss, and left corneal ulceration with chalky white iris deposits.

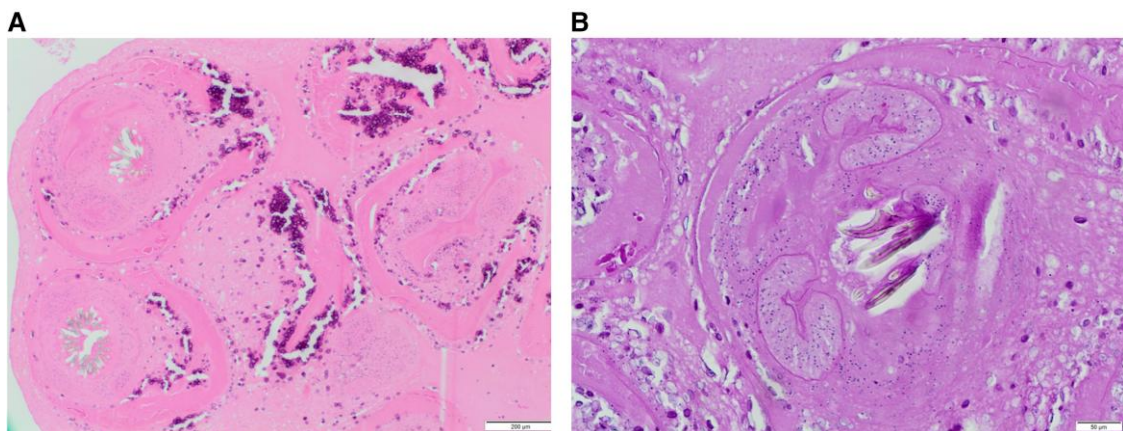


Figure 2. Histological examination of brain biopsy tissue (case 2) (A) and at higher resolution (B) confirmed the presence of parasitic infection with numerous scoleces within the cyst and rostellum with hooklets.

lymphocytic inflammation and foamy macrophages. The appearances were of a parasitic infection, favoring coenurosis over neurocysticercosis given the presence of multiple scoleces per cyst. Steroids were tapered over 2 weeks, with good clinical recovery. Coenurosis was confirmed by WGS identification of *Taenia serialis* from resected tissue.

DNA Sequencing

Tissue samples were mechanically disrupted, and the supernatant was boiled then mechanically disrupted again. Cellular DNA was purified without enrichment using magnetic beads. Library preparation was performed using the SQK-LSK109 genomic DNA ligation sequencing kit (Oxford Nanopore, Oxford, UK). DNA sequencing was performed using a FLO-MIN106 R9.4.1 GridION flow cell with MiniKNOW sequencing software (Oxford Nanopore, Oxford, UK) ([Supplementary Methods](#)).

For case 2, tissue also underwent mitochondrial 12S ribosomal DNA (rDNA) polymerase chain reaction (PCR) in triplicate, as previously described [5], using specific probes for *Echinococcus multilocularis*, *E. granulosus sensu lato*, and *Taenia* species, with Sanger sequencing of the PCR product (~180 bp) to identify *Taenia* species.

Sequencing Analysis

For case 1, sequencing reads were base-called using Guppy, version 3.0.6 (Oxford Nanopore, Oxford, UK), and analyzed in real time using the in-house workflow, CRuMPIT [6]. Reads were taxonomically classified using Centrifuge, version 1.0.4-beta [7]. Minimap2 [8] was used to map reads classified as *M. leprae* to a reference genome, NC_002677.1, for phylogenetic comparison with published sequences ([Supplementary Methods](#)).

For case 2, reads were base-called using Guppy, version 3.0.6, and classified using Kraken2, version 2.0.8 [9], with default parameters. Two custom Kraken2 databases were used ([Supplementary Table 1](#)). The first comprised the human genome (GRCh38.p12) and all 173 complete, repeat-masked worm genomes from WormBase ParaSite, version 14 [10]. To increase resolution of *Taenia* species, a second database comprised the human genome and 16 complete (circular) mitochondrial genomes from the genus *Taenia*, plus 4 *Taenia* genomes recently reclassified into the *Hydatigera* and *Versteria* genera. Read classification was checked by mapping to reference using minimap2 [8] with default parameters.

After removal of sequencing reads classified as human [6], the raw sequencing data have been deposited in the European Nucleotide Archive (accession number PRJEB45350).

RESULTS

WGS of DNA extracted from the lymph node biopsy of case 1 confirmed the presence of *M. leprae*. Of 3.4 million sequencing

reads, 61 637 (1.8%) belonged to bacterial species (98.2% of reads were human), and among bacterial reads, 61 449 (99.7%) were classified as *M. leprae*. No reads were classified as another mycobacterial species, excluding mixed mycobacterial infection. Mycobacterial reads gave almost complete *M. leprae* genome coverage, with 99.9% reference genome coverage to 18-fold depth. Variant analysis showed that this isolate was highly similar to those identified in Asia, Africa, and South America ([Supplementary Figure 2](#)).

Antimicrobial susceptibility was predicted by comparison with a *M. leprae* reference and published catalogues of resistance-conferring mutations [11]. No variants were identified in RNA polymerase B (*rpoB*) compared with the wild-type, suggesting rifampicin susceptibility. Within DNA gyrase subunits A and B (*gyrA* and *gyrB*), 1 single nucleotide polymorphism (SNP) in *gyrA* at T1136C was found compared with wild-type. This mutation, which is not known to confer quinolone resistance [11], is predicted to encode a nonsynonymous mutation from leucine to proline at amino acid 379. This region is predicted to be excised in post-translational processing [12], so we predict no effect on fluoroquinolone susceptibility.

For case 2, mitochondrial rRNA sequencing supported a classification of *Taenia* spp. Sequencing of a 177-bp amplicon was compared with *Taenia* species in the NCBI database and identified *T. serialis* with 99.4% sequence identity or *T. multiceps* with 97.7% sequence identity.

WGS for case 2 generated 2 million ONT reads of total length 3.2×10^9 bp (80% of which were >1 kb and 14% >5 kb). Classification against the custom worm genome database identified *Taenia multiceps* as the best-hit candidate, supported by 13 109 reads, 0.63% of the total (98.35% human reads) ([Supplementary Table 2A](#)). With the increased resolution of a *Taenia*-specific database constructed from the available set of mitochondrial genomes (n = 21) ([Supplementary Table 1](#)), the best hit was *T. serialis*. One hundred eighty-eight reads were classified as *T. serialis*, representing ~14-fold coverage of the mitogenome ([Supplementary Table 2B](#)). No reads were classified to the *T. multiceps* mitogenome. Classification was confirmed by alignment of sequencing reads to the *T. serialis* and *T. multiceps* mitogenomes; 100% of reads could be aligned to *T. serialis*, but only 82% to *T. multiceps*.

DISCUSSION

We demonstrate the application of WGS direct from tissue to support the challenging clinical diagnosis of both bacterial and parasitic pathogens that cannot easily be cultured, to inform treatment in real time, and to enhance our understanding of infectious disease etiology and epidemiology.

In multibacillary leprosy, WGS was able to predict antimicrobial susceptibility, directly informing treatment in the context of previous treatment, which increases risk of antimicrobial

resistance [11]. Further work is needed to assess the sensitivity in paucibacillary disease.

Species-level diagnosis of the rarer parasitic infection *T. serialis* in the second case provides insights into disease etiology, as this organism has only once been reported as a cause of human neurocoenurosis [13], the usual reported cause being *T. multiceps*. *T. multiceps* and *T. serialis* larvae cannot reliably be distinguished morphologically, so it is possible that *T. serialis* causes a larger proportion of cases [14].

Importantly, we used a portable sequencing platform, potentially employable in resource-limited settings, as demonstrated in the COVID-19 pandemic [15]. By simultaneously yielding both diagnostic and antimicrobial susceptibility data without specialist PCR or culture-based techniques, this powerful, portable tool may simultaneously aid diagnosis, provide antimicrobial resistance testing and surveillance [16, 17], and delineate transmission networks [18] for difficult-to-culture pathogens like *M. leprae*. Both cases highlight the potentially important advances that modern WGS methods may bring to microbiological diagnosis of ancient pathogens.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Financial support. The work was supported the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre (BRC).

Disclaimer. The views expressed in this publication are those of the authors and not necessarily those of the NHS or the National Institute for Health Research.

Potential conflicts of interest. All authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Patient consent. The written consent of both patients whose cases are discussed here was obtained. The design of the laboratory work did not

require ethical review as it was a laboratory method development focusing on pathogen genomic data from routinely collected samples (sequencing reads identified as human were counted then discarded).

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