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

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Introduction

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 centralised.[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 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 six months, developing tender red swellings which ulcerated and bled; and five months' painful, watering eyes, with blurred vision in the left eye. She had lost seven kilograms 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 previously well. She was born in a Maritime Southeast Asian nation, 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, abdominal 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-necrotising granulomas and abundant mycobacteria. Molecular testing for *Mycobacterium tuberculosis* was negative.

Lepromatous leprosy and Type 2 reaction (Erythema Nodosum Leprosum) was suspected. Ophthalmological examination revealed a left corneal ulcer with hypoesthesia; and bilateral subepithelial 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 multi-drug therapy for presumed leprosy (but had not herself been informed of this diagnosis). *Mycobacterium leprae* infection was confirmed by WGS of lymph node tissue thirteen days after biopsy. Treatment was started with rifampicin, ofloxacin and minocycline.

Clinical case 2

A 54-year-old woman presented to hospital in Ghana with right-sided sensory seizures. She had no previous medical history. MRI brain showed a single, left parietal, rim enhancing lesion with significant surrounding oedema (Figure S1). 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 oedema after 3 months, 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 reactive brain parenchyma, with perivascular lymphocytic inflammation and foamy macrophages. The appearances were of a parasitic infection, favouring coenurosis over neurocysticercosis given the presence of multiple scoleces per cyst. Steroids were tapered over two 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 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, using MiniKNOW sequencing software (Oxford Nanopore, Oxford, UK; see Supplementary Methods).

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

Sequencing analysis

For case 1, sequencing reads were base-called using Guppy v3.0.6 (Oxford Nanopore, Oxford, UK) and analysed in real time using the in-house workflow, CRuMPIT.[6] Reads were taxonomically classified using Centrifuge v1.0.4-beta.[7] Minimap2[8] was used to map reads

classified as *M. leprae* to a reference genome, NC_002677.1 for phylogenetic comparison to published sequences (see supplementary methods).

For case 2, reads were base-called using Guppy v3.0.6 and classified using Kraken2 v2.0.8[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 v14[10]. To increase resolution of *Taenia* species, a second database comprised the human genome and 16 complete (circular) mitochondrial genomes from genus *Taenia,* plus 4 *Taenia* genomes recently reclassified into the *Hydatigera* and *Versteria* genuses. 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 is deposited in the European Nucleotide Archive (accession number PRJEB45350).

Results

WGS of DNA extracted from 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 this isolate was highly similar to those identified in Asia, Africa and South America (Figure S2).

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*) one single nucleotide polymorphism (SNP) in *gyrA* at T1136C was found compared to wild type. This mutation, which is not known to confer quinolone resistance, [11] is predicted to encode a non-synonymous 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 177bp 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 $3.2x10^9$ bp (80% of which were >1 kb and 14% >5kb). 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*. 188 reads were classified as *T. serialis*, representing approximately 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 *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 aetiology 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 aetiology, 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.

Funding

The work was supported the NIHR Biomedical Research Centre, Oxford. 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

The authors have no conflicts of interest to disclose.

Patient Consent Statement

The written consent of both patients whose cases are discussed here was obtained. The design of 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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Figures

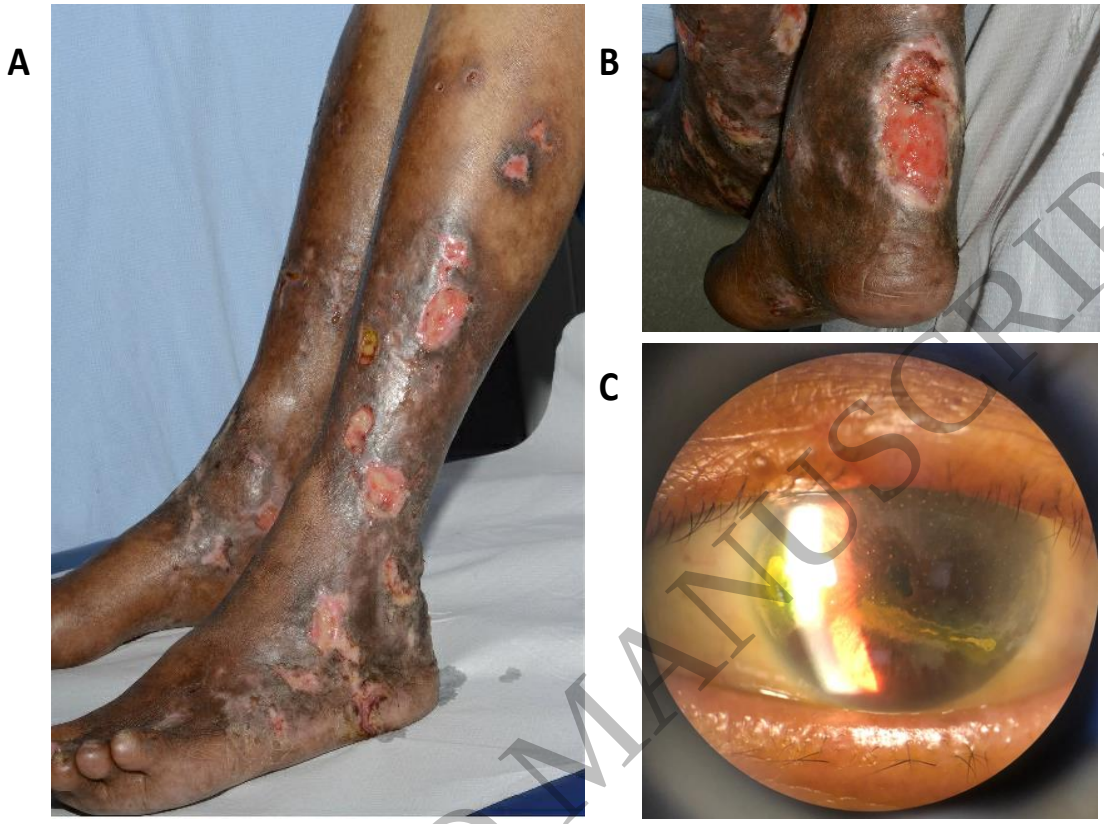

Figure 1 – Clinical images from Case 1: Multibacillary leprosy with Type 1 Reaction (Erythema Nodosum Leprosum). The patient presented with multiple irregular deep ulcers over both lower limbs (1a, 1b). Examination of left eye showing thickened eyelid, eyelash loss, and left corneal ulceration with chalky white iris deposits (1c).

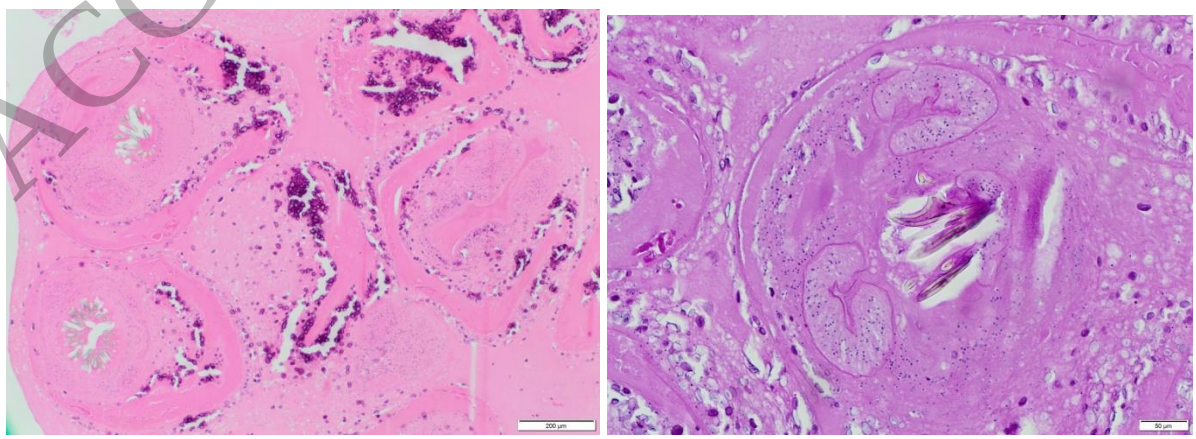

Figure 2. Histological examination of brain biopsy tissue (Case 2) confirmed presence of parasitic infection with numerous scolices within the cyst, and rostellum with hooklets.