Coccidioidal Meningitis in New York traced to Texas by Fungal Genomic Analysis

Bridget M Barker PhD¹, Sujatha Rajan MD², Marcus de Melo Teixeira PhD¹,³, Michelle Sewnarine MD², Chandler Roe MS¹,⁴, David M Engelthaler PhD⁴, & John N Galgiani MD⁵.

¹Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff Arizona; ²Cohen Medical Center, Long Island New York; ³Faculty of Medicine, University of Brasília, Brasília-DF, Brazil; ⁴Translational Genomics Research Institute, Flagstaff Arizona; and ⁵Valley Fever Center for Excellence and Department of Medicine, University of Arizona College of Medicine, Tucson, Tucson Arizona.

Correspondence and reprint requests:

John N Galgiani MD
Valley Fever Center for Excellence
University of Arizona
PO Box 4073
PO Box 245215
Tucson AZ 85724
Tel: 520-626-4968
Fax: 520-626-4971
Email: spherule@u.arizona.edu

Alternate correspondent:

Bridget M Barker PhD
Pathogen and Microbiome Institute
Northern Arizona University
PO Box 4073
Flagstaff AZ, 86011
Tel: 928-523-6074
Email: bridget.barker@nau.edu
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Abstract. [word count = 50]

A child developed hydrocephalus. Sixteen months later, it was discovered to be a complication of coccidioidal meningitis. The infection’s source was uncertain until genomic analysis of the fungal isolate identified its origin to be a visit to Beeville Texas.

Improved national reporting of cases of coccidiodomycosis might reduce diagnostic delays.
Introduction.

Coccidioidomycosis, also known as San Joaquin Valley fever, is a Western Hemispheric endemic fungal disease. In the United States, most reported infections originate from exposures in Arizona and California, but unexpected infection sources are continuing to be found elsewhere in the western USA [1]. Infections are also recognized outside of known endemic areas [2], usually associated with recent travel to and exposure in known endemic regions. Less frequently, they are reactivation during immunosuppression from a distant past infection or from transplantation of infected tissue. Rarely, non-endemic coccidioidomycosis is the result of fomite transmission [3]. Although designated a reportable disease, surveillance is passive and not all states participate. For example, current statistics maintained by the CDC show no new cases of coccidioidomycosis occurring in Texas although publications from last century have demonstrated western portions of the state are endemic [4, 5].

We report here an otherwise healthy child living in Long Island NY who was found to have coccidioidal meningitis with recent travel only to non-endemic regions of California. Subsequent investigation identified past travel years earlier to both California and Texas. Whole-genome sequencing of the patient’s fungal isolate enabled precise determination of the source of infection. This case further demonstrates the power of fungal genomics to assist in the epidemiologic understanding of endemic fungal diseases. It also underscores that more comprehensive surveillance of coccidioidomycosis might benefit persons who live in or visit endemic regions.
Case Report.

A 17 month-old boy who lived with his parents in Long Island, New York, developed headache, ataxia, and lethargy and was found to have hydrocephalus in July 2013. In the prior several months, he had been more irritable than usual but no specific diagnosis was made. Lumbar CSF showed 10 RBC and 31 WBC per mm$^3$ (2% neutrophils, 58% lymphocytes, 36% monocytes, and 2% eosinophils), glucose and protein of 21 and 78 mg/d, respectively. A ventriculo-peritoneal shunt was placed at which time ventricular fluid showed 23 RBC and 21 WBC per mm$^3$ (3% neutrophils, 61% lymphocytes, 34% monocytes, and 2% eosinophils), glucose and protein of 30 and 77 mg/dL, respectively. Cultures for bacteria and fungi of both fluids were negative.

In October 2014, headache recurred, magnetic resonance imaging of the brain revealed recurrent enlargement of the ventricles, and the proximal portion of the shunt was revised surgically. At that time, CSF from the reservoir demonstrated 9 WBC per mm$^3$, glucose and protein of 41 and 11 mg/dL, respectively. Gram-stain and bacterial cultures were negative. The patient was discharged after an unremarkable post-operative stay, but he was readmitted one day later with worsening headache and lethargy. Ventricular fluid now showed 86 WBC per mm$^3$, glucose and protein concentrations unchanged, but the gram stain showed a few large gram-variable filamentous structures with box car-like structures that stained poorly with fungal stain (Supplemental Figure 1). These findings prompted the initiation of intravenous amphotericin B and voriconazole, and the entire shunt was surgically removed. Cultures of the CSF and an intra-abdominal cystic fluid collection obtained during surgery both grew *Coccidioides* spp. as initially determined by genetic hybridization (GenProbe, San...
Diego CA) and was subsequently identified as *C. posadasii* by PCR (Sudha Chaturvedi, personal communication). Treatment was switched to oral fluconazole which has been continued to the present. The child returned to good health and has had no further complications with nearly four years of follow-up.

The time-line for the patient’s travel history in relation to the original ventriculoperitoneal shunt and the subsequent complications when coccidioidomycosis was identified is shown in the Figure. The patient traveled to Texas and California before being diagnosed with hydrocephalus. Past travel in California included a trip along the coast from Los Angeles to the San Francisco bay area. The route was through Santa Barbara and San Luis Obispo Counties, regions recognized as endemic for *Coccidioides immitis*. However, because the isolate was identified as *C. posadasii*, additional investigation was warranted.

**Genomic Analysis.**

Fungal cultivation and DNA extractions were performed under BSL-3 conditions at the University of Arizona. DNA yield was assessed using spectrophotometry on the NanoDrop® ND-1000 system (Thermo Fisher Scientific). Sequencing libraries were prepared using the Kapa Biosystems kit (Kapa Biosystems, Wilmington, MA) and 1μg of DNA was used for input material. Libraries were multiplexed using 8-bp indexes and quantified using quantitative PCR (qPCR) in a 7900HT system (Life Technologies Corporation, Carlsbad, CA) using a Kapa library quantification kit (Kapa Biosystems, Wilmington, MA). Libraries were sequenced on an Illumina MiSeq instrument in a 2 X 300 mode (Illumina, San Diego, CA). Reads were de-multiplexed using in-house scripts and poor quality reads were removed from subsequent analysis. The raw sequence
data were deposited at Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under following accession number: SRR7586647. The SNP matrix was obtained using the NASP toolkit [6]. Phylogenomic analysis was conducted using Maximum Likelihood (ML), using previously published genomes and methods [7]. The ML tree (Supplemental Figure 2) reveals that the isolate obtained from the CSF clusters within C. posadasii, confirming the PCR results. The isolate also groups with other isolates from Texas, which aligns with the child’s past trips to Beeville Texas. Although Texas does not report cases of coccidioidomycosis to the CDC, a report from 1970 had previously identified a cluster of infections occurring in the same region of Texas [5].

Discussion.

This case illustrates how genomic analysis has become increasingly relevant to understanding the source of unexpected and otherwise cryptic appearance of coccidioidomycosis. All strains in the genus Coccidioides were considered of the same species, C. immitis, until it was discovered to contain two distinct clades, one of which is now designated C. immitis and the other as C. posadasii [8]. Clinical isolates of Coccidioides spp. normally are identified only to the level of genus, as was done initially for this patient. Genus identification provides no insight into where the fungus was acquired. However, more detailed genetic analysis is increasingly useful to assist with understanding cases such as this one [9]. The genomic sequence of the isolate was most similar to other isolates from Texas, including a Guatemalan patient (strain 730334, Supplemental Figure 2) that also had travel history to the same state [7]. This confirms that the most parsimonious source of infection was indeed in Beeville, TX. Although the patient was again in Beeville following his original exposure, most likely
December 2012, there is no reason to believe that this return visit in 2013 had any bearing on his illness.

This case also illustrates several important clinical details. First, coccidioidal meningitis in children, unlike in adults, frequently presents as unexplained hydrocephalus, and often cultures of ventricular fluid yield Coccidioides spp. [10] as did fungal cultures at the time of the shunt revision. Second, as indicated by the lumbar and ventricular fluid analysis when hydrocephalus was first discovered, ventricular fluid abnormalities in coccidioidal meningitis are less abnormal than those of lumbar CSF [11]. This is because CSF is secreted principally from the choroid plexuses, moves from the ventricular cavities through foramina into the subarachnoid space, and is reabsorbed by the arachnoid villi. This flow through an inflamed meninges results in progressively increasing abnormalities. Third, the son’s mother, who had grown up in Beeville, TX, was unaware of coccidioidomycosis in her region even though older medical literature makes it clear this area was endemic [5]. Without this knowledge, the general differential for unexplained pediatric hydrocephalus would include congenital malformations, infections, tumors, or an intraventricular hemorrhage but not coccidioidal meningitis [12]. Although the CDC designates coccidioidomycosis as a reportable disease, reporting is dependent upon the participation of individual state departments of health. A recent report documented delays of up to four and a half years in accurately diagnosing patients with coccidioidomycosis who presented in states not known to be endemic for this disease [2]. It is possible that if Texas participated in the reporting of this disease, this child’s infection might have been identified in a more timely manner. In
any case, a detailed travel history may be essential in evaluating hydrocephalus in the presence of abnormal CSF studies.
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Figure Legend. Timeline for the patient’s travel in relation to illness.