

## Valley Fever: Finding New Places for an Old Disease: *Coccidioides immitis* Found in Washington State Soil Associated With Recent Human Infection

Anastasia P. Litvintseva,<sup>1</sup> Nicola Marsden-Haug,<sup>2</sup> Steven Hurst,<sup>1</sup> Heather Hill,<sup>3</sup> Lalitha Gade,<sup>1</sup> Elizabeth M. Driebe,<sup>4</sup> Cindy Ralston,<sup>3</sup> Chandler Roe,<sup>4</sup> Bridget M. Barker,<sup>4</sup> Marcia Goldoft,<sup>2</sup> Paul Keim,<sup>4</sup> Ron Wohrle,<sup>2</sup> George R. Thompson III,<sup>5</sup> David M. Engelthaler,<sup>4</sup> Mary E. Brandt,<sup>1</sup> and Tom Chiller<sup>1</sup>

<sup>1</sup>Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia; <sup>2</sup>Washington State Department of Health, Shoreline and Tumwater, and <sup>3</sup>Benton-Franklin Health District, Kennewick, Washington; <sup>4</sup>Translational Genomics Research Institute, Flagstaff, Arizona; and <sup>5</sup>Coccidioidomycosis Serology Laboratory, University of California, Davis

**We used real-time polymerase chain reaction and culture to demonstrate persistent colonization of soils by *Coccidioides immitis*, an agent of valley fever, in Washington State linked to recent human infections and located outside the endemic range. Whole-genome sequencing confirmed genetic identity between isolates from soil and one of the case-patients.**

**Keywords.** *Coccidioides*; valley fever; soil detection; WGS typing.

The soil-dwelling fungi *Coccidioides immitis* and *Coccidioides posadasii* are etiologic agents of coccidioidomycosis (valley fever), which is common in the southwestern United States, Mexico, and South America [1]. Unlike most other human pathogenic fungi, *Coccidioides* can infect immunocompetent individuals and cause a broad spectrum of diseases, ranging from asymptomatic infection to mild respiratory illness to severe life-threatening infections [1]. Our current understanding of the geographic distribution of *Coccidioides* is based on the results of skin testing conducted in the 1950s and is likely to underestimate prevalence and distribution of these pathogens in the Western hemisphere [1–3].

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Correspondence: Anastasia P. Litvintseva, PhD, Mycotic Diseases Branch, Centers for Disease Control and Prevention, 1600 Clifton Rd, MS G-11, Atlanta, GA 30333 (frq8@cdc.gov).

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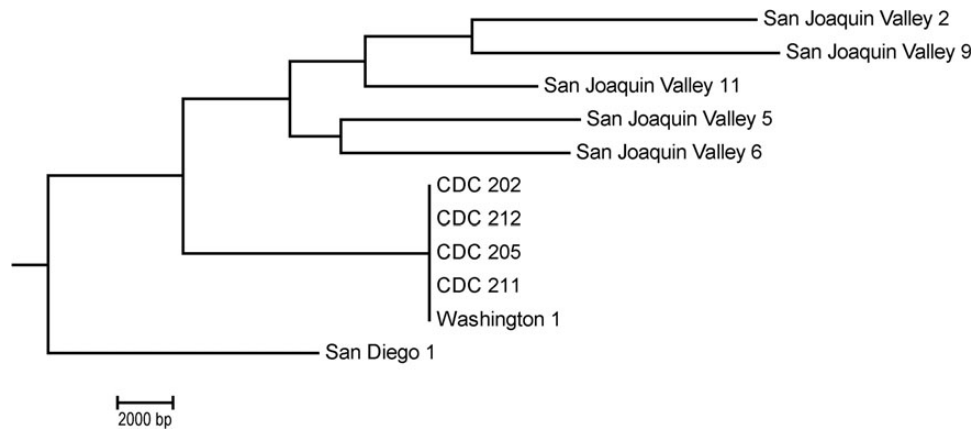
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In 2010, the Washington State (WA) Department of Health identified 3 cases of coccidioidomycosis in eastern WA, an area not previously known to have endemic disease [4]. Although epidemiologic data suggested that *Coccidioides* might have been acquired from the local environment, all 3 case-patients had traveled to endemic regions several years prior to infection; therefore, the possibility of reactivation of latent infection could not be excluded [4]. To determine whether these infections were acquired in WA, we collected soil samples from 2 sites epidemiologically linked to possible exposures, and used a novel real-time polymerase chain reaction (PCR) assay and culture to confirm the presence of *C. immitis* in soil.

To identify sites for environmental sampling, 2 of 3 case-patients were interviewed about their exposures to soil and accompanied investigators to the specific locations in Benton County, WA, where these activities occurred. Patient 1, who developed pneumonia, reported regularly playing and digging in a dirt canyon at site 1 located on public land near a residential complex. Patient 2 suffered a knee wound infection after falling from his all-terrain vehicle (ATV) at site 2, a dirt track at a public park used for ATV riding. From these 2 sites, 22 soil samples were collected in November 2010, and 25 soil samples were collected again in April 2014 (Supplementary Table 1) and stored at 2°C–11°C for either 3 years or 5 days prior to analysis.

Soils were tested using the “CocciDx” real-time PCR assay developed at the Translational Genomics Research Institute (Supplementary Table 2). From the 2010 soil samples, *Coccidioides* DNA was detected in 1 sample from site 1 and 5 samples from site 2. *Coccidioides* DNA was also detected in soil samples collected in 2014, >4 years after original collection: 5 of 10 samples collected from site 1 and 11 of 15 samples from site 2 were positive (Supplementary Table 1). No amplification was observed in any of the negative control soils from Atlanta, Georgia, or control fungal cultures after 45 cycles (Supplementary Table 3).

All soil manipulation procedures were conducted under Biosafety Level 3 conditions. For culture, soil samples were mixed with phosphate-buffered saline for 2 hours and allowed to settle, and 100- $\mu$ L aliquots of the soil suspension were plated on a modified yeast extract medium (yeast extract 15 g/L, Hutner’s Trace Elements 2 mL/L, cycloheximide 0.5 g/L, chloramphenicol 0.05 g/L, gentamicin 0.05 g/L, agar 20 g/L) and incubated at 37°C for 5 days. White, fuzzy colonies were selected and subjected to internal transcribed spacer 2 sequencing for molecular confirmation. From site 2, 15 isolates of *C. immitis* were recovered from 5 soil samples collected in 2010, and 1 isolate was



**Figure 1.** Phylogenetic analysis of genomes of isolates from the patient (Washington 1), Washington environment (CDC 202, CDC 205, CDC 211, and CDC 212), and unrelated *Coccidioides immitis* strains (n = 6) from California visualized by maximum parsimony method and rooted at midpoint. Abbreviation: CDC, Centers for Disease Control and Prevention.

recovered from 2014 soil, confirming the presence of viable isolates in soil after 4 years. Six isolates were recovered from the surface, 4 from inside a snake/rodent burrow, and 6 isolates from soil 6 inches below the surface collected a few feet away from patient 2's ATV crash site. All 6 of those soil samples were also positive with the CocciDx assay (Supplementary Table 1).

Initial specificity tests of CocciDx PCR suggest that this assay is specific to *Coccidioides*. However, because this assay relies solely on specific hybridization with a TaqMan probe, and the soil environment may contain previously uncultured fungi that can potentially cross-react with the assay, its specificity needs to be further evaluated. Nevertheless, we are confident in our interpretation of the PCR results in this study, because PCR results were confirmed by culture. Furthermore, isolation of cultures from the patient and the soils occurred in separate laboratories (University of California, Davis and the Centers for Disease Control and Prevention, respectively), preventing possible contamination effects. Isolation of *C. immitis* from soils is considered challenging and historically a mouse inoculation method has been used to improve isolation efficiency [5]. Here, we modified yeast extract agar [6] to isolate *C. immitis* from WA soils. Both novel real-time PCR and culture methods need to be further evaluated using soils from other areas to assess their utility for the environmental detection of *Coccidioides*.

Whole-genome sequencing (WGS) was used to evaluate relationships among 4 representative isolates from soil, 6 unrelated strains from California, and a clinical isolate obtained from a ruptured inguinal lymph node of patient 2 (Washington 1). WGS was conducted using an Illumina MiSeq platform resulting in 48 times average sequencing depth and 76% coverage breadth of genomes. Genomes were assembled using the San Diego 1 strain as a reference and deposited at the National

Center for Biotechnology Information under the BioProject accession number PRJNA245906.

The genome of *C. immitis* is approximately 28 Mb in size; no more than 3 single-nucleotide polymorphisms (SNPs) differentiated DNA from the Washington 1 patient isolate and any of the WA soil isolates that were sequenced, with a total of 10 SNPs among all members of this group. Conversely, >21 000 SNPs were identified between the WA strains and *C. immitis* strains from other regions (Figure 1). The population genomic structure of *Coccidioides* is well established; natural isolates of *C. immitis* are very diverse and thousands of SNPs separate strains from the same geographic area [7]. Our results indicate that although WA isolates form a distinct clade on the phylogenetic tree, they are more related to strains collected in the San Joaquin Valley compared with a single strain from San Diego (Figure 1). Near-genomic identity observed among genomes of 5 sequenced WA strains indicates the recent common origin of these isolates. Similar degrees of genomic relatedness were observed when 3 individual *C. immitis* genomes from 3 transplant recipients sharing a common organ donor were compared in a case of contaminated transplant organs [8]. Our WGS results provide a direct link between the Washington 1 patient isolate and its environmental source.

There are at least 4 possible explanations for the presence of *C. immitis* in WA. First, because *C. immitis* was isolated from the ATV track soil, it is possible that it was transported from another endemic area on ATV vehicles and became established in an area with similar climate and ecology. Fomite transmission of *Coccidioides* has been reported previously [9]. Second, recent extreme weather events and/or climate change might have contributed to *C. immitis* introduction to WA. Specifically, droughts often result in massive production of dust, which has been shown to transmit fungal spores [10, 11]. Recent extensive

droughts that affected California, Oregon, and WA might have contributed to the expansion of the *C. immitis* range. Third, it is also possible that *Coccidioides* has been present in WA for an extended time, but because of low human population density in eastern WA, sporadic distribution of the pathogen, and lack of awareness among physicians, human cases of coccidioidomycosis have not been previously recognized in WA. This hypothesis is supported by the fact that animal cases of coccidioidomycosis have been described in WA [4], although no exposure history is available for these cases. Fourth, other factors, such as expanding rodent or other animal populations, may have also contributed to the expansion of *C. immitis*. More research using molecular methods of detection and better epidemiological surveillance is necessary to determine prevalence of *Coccidioides* in WA and the surrounding states.

Our results have significant public health implications. Clinicians, diagnostic laboratories, and the public need to be aware that this disease can be contracted outside the traditionally established endemic areas. More research is essential to determine the prevalence of *Coccidioides* in WA and other areas that border the historically defined endemic areas. These 3 cases of coccidioidomycosis may represent a small proportion of such cases in WA. Epidemiological data from the endemic areas suggest that up to 60% of infected people remain asymptomatic and <5% develop severe or disseminated disease that requires hospitalization, suggesting that there might be additional unidentified cases of valley fever in WA. Recurrent detection of *Coccidioides* DNA and isolate recovery from the same soils 4 years after infection indicates persistent colonization of WA soils and suggests that more infections may occur in this region.

### Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (<http://cid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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**Disclaimer.** The use of product names does not imply their endorsement by the US Department of Health and Human Services. The finding and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

**Potential conflicts of interest.** P. K. and D. M. E. have a financial interest in DxNA, the licensee of the real-time PCR assay described in this manuscript. All other 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.

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