



# The Association between *Coccidioides immitis* and Rodent Habitats in Washington State Remains Unresolved

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On 22 December 2021, we published a research article describing the distribution of *Coccidioides immitis* in soil in Washington State (1). There, we used a systematic sampling approach, *Coccidioides*-specific reverse transcriptase PCR (RT-PCR), amplicon sequencing, and soil chemical analyses to describe the distribution of *C. immitis* in soil. We identified soil chemical and microbiological signatures associated with the presence of *Coccidioides* DNA and demonstrated that the same strain can colonize a 46,000-m<sup>2</sup> area for 6 years. We also reported no association between rodent habitats and *C. immitis*, as equal proportions of *Coccidioides*-positive samples were detected in rodent burrows and in the surrounding soils.

After publication of the article, it was brought to our attention that the soil sampling approach used in our study was not well suited for testing the association between *C. immitis* and rodent habitats because different strategies were used for collecting soil inside and outside rodent burrows. Specifically, because of the narrow entrances, single-soil samples were collected from burrows. However, for nonrodent burrow samples, three subsamples (“scoops”) were collected from each 1-m<sup>2</sup> plot and mixed into one composite sample to obtain representative samples for chemical analysis. The uneven distribution of *Coccidioides* spp. in soil has been described (2, 3), and it is possible that not all scoops mixed into a composite sample contained *Coccidioides* DNA. However, even if only one of the three scoops in a composite sample was positive, the entire sample would test positive, leading to an inflated number of positives and making the direct comparison between soils from inside and outside rodent burrows uninformative.

To estimate the degree of uncertainty introduced by this soil sampling approach, we performed a sensitivity analysis and demonstrated that for the observed “no association” relationship between *C. immitis* and rodent burrows to remain true at a 0.05 significance level, at least 51% of the positive composite samples must have comprised at least two positive scoops (this number could have been lower if some composite samples included three positives). Considering the widespread prevalence of *C. immitis* at the site, multiple positive scoops within the same plot can be expected; however, we agree that the presented data are not able to appropriately look at this association.

It is important to note that several findings, which were unaffected by the sampling design, support the ability of *C. immitis* to propagate in soils without rodent activity. These findings include the following: (i) the observed difference in chemical composition between *Coccidioides*-positive and negative soils, and (ii) the frequent detection of *C. immitis* DNA in areas along the transects without rodent activity (56% of soils within the 4-m radius were positive, despite the presence of only a single rodent burrow on the outskirts of this area). Furthermore, in the laboratory, *C. immitis* grew on soil as a sole source of nutrients.

In summary, we acknowledge that our conclusion about the lack of an association between the presence of *C. immitis* and rodent habitats is insufficiently supported by the data and requires additional testing. We published this letter to clarify this point.

**Editor** Aaron P. Mitchell, University of Georgia

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The authors declare no conflict of interest.

For the article discussed, see <https://doi.org/10.1128/mSphere.00598-21>.

**Published** 11 July 2022

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