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Journal

Medical Mycology, 57(Supplement_1)

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Publication Date

2019-02-01

DOI

10.1093/mmy/myy039

Peer reviewed

ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2019, 57, S16–S20
doi: 10.1093/mmy/myy039
Review Article

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The endozoan, small-mammal reservoir hypothesis and the life cycle of *Coccidioides* species

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Received 6 February 2018; Revised 25 April 2018; Editorial Decision 1 May 2018

Abstract

The prevailing hypothesis concerning the ecology of *Coccidioides immitis* and *C. posadasii* is that these human pathogenic fungi are soil fungi endemic to hot, dry, salty regions of the New World and that humans and the local, small-mammal fauna are only accidental hosts. Here we advance an alternative hypothesis that *Coccidioides* spp. live in small mammals as endozoans, which are kept inactive but alive in host granulomas and which transform into spore-producing hyphae when the mammal dies. The endozoan hypothesis incorporates results from comparative genomic analyses of *Coccidioides* spp. and related taxa that have shown a reduction in gene families associated with deconstruction of plant cell walls and an increase in those associated with digestion of animal protein, consistent with an evolutionary shift in substrate from plants to animals. If true, the endozoan hypothesis requires that models of the prevalence of human coccidioidomycosis account not only for direct effects of climate and soil parameters on the growth and reproduction of *Coccidioides* spp. but also consider indirect effects on these fungi that come from the plants that support the growth and reproduction of the small mammals that, in turn, support these endozoic fungi.

Key words: *Coccidioides*, coccidioidomycosis, life cycle, endozoan, animal reservoir.

This short article presents the following hypotheses: (*i*) that *Coccidioides* species, *C. immitis* and *C. posadasii*, are endozoans, that is, they live as spherules in granulomas formed by their small mammal hosts without causing apparent disease, (*ii*) that when the host dies, the fungi are released from the granulomas, the spherules or endospores convert to hyphae and the hyphae use the substrate provided by the dead mammal to produce abundant clonal propagules, that is, arthroconidia, and (*iii*) these conidia are inhaled by naive hosts thus initiating new infections and continuing the life-cycle (Fig. 1 and 2). The small-mammal reservoir, or endozoan, hypothesis owes much to the discovery of fungi that live as endophytes in plants without causing apparent symptoms, only to grow and reproduce with the plant or plant part dies. The key point underpinning the endozoan hypotheses is the advantage provided *Coccidioides* species by being inside the mammal when it dies, thus gaining access to the nutritive substrate as soon as it becomes available. Note that this hypothesis does not preclude growth of the fungi into the surrounding soil in search of organic carbon while they are supported by the relatively larger amounts of organic matter in an animal carcass or midden. Readers are directed to a recent review of the *Coccidioides* life cycle[1] and a different view of the association of *Coccidioides* with small mammals.[2]

The hypothesis that small mammals provide the reservoir for *Coccidioides* species was developed by Chester Emmons[3] from discoveries of granulomas in the lungs of small mammals trapped in Arizona in the 1930s and 1940s[4] and identification by cultivation from the granulomas of *Coccidioides*.[5] Emmons' research followed on the discovery that what had been considered to be an invariably fatal disease, *Coccidioides* granuloma, was caused by the same fungus responsible for the more benign San Joaquin Fever.[6] This discovery initiated epidemiological studies of coccidioidomycosis[7] and ecological studies of the fungi. A decade or more after Emmons' research in Southern Arizona, Egeberg and Ely, working in western Kern County, California, showed that *C. immitis* was twice as likely to be

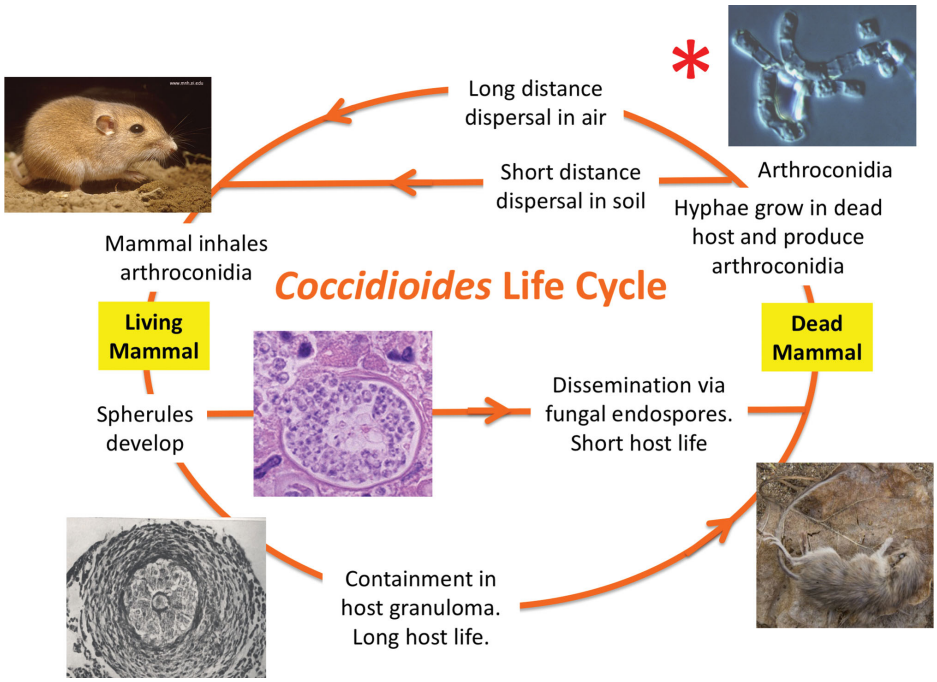

Figure 1. Endozoan-based Life cycle of *Coccidioides* species. Beginning at the asterisk (∗), arthroconidia travel from the hyphae that produced them short distances among small mammals in burrows, or longer distances above ground, to be inhaled by uninfected animals. In the lungs, the arthroconidia convert to spherules and are either controlled by the host cell-mediated immune reaction or develop endospores, which disseminate to produce grave disease. The infected animal dies, either from disseminated coccidioidomycosis or from other causes and, in either case, living *Coccidioides* present in the animal, now freed from the action of the host immune system and living at lower temperatures, convert to hyphae. The hyphae grow through the dead animal and then produce abundant arthroconidia, which initiate a new cycle of life for the fungus. Photos: Living San Joaquin Pocket Mouse, *Perognathus inornatus*, Moose Petersen, http://www.mnh.si.edu/mna/images/images/san_joaquin_pocket_mouse_thumb.jpg ; *Coccidioides* spherules and endospores, Dr. Edward Klatt, WebPath's Internet Pathology Laboratory for Medical Education; Granuloma constraining *Coccidioides*[3]; Dead *Perognathus longimembris*, Little Pocket Mouse, Jonah Evans, Texas Parks and Wildlife Department, http://www.inaturalist.org/photos/407437. This Figure is reproduced in color in the online version of *Medical Mycology*.

recovered from soil collected from rodent burrows as from other soil, causing them in 1956 to echo Emmons' hypothesis about a small rodent reservoir.[8] However, a study a year later of a Native American midden in far eastern Kern County, California, by Swatek and colleagues quashed the rodent hypothesis when massive sampling of soil and small mammals surrounding an infested midden failed to turn up a single *Coccidioides* isolate.[9] The midden in question was near Inyokern, California, which is east of the San Joaquin Valley on the eastern slope of Sierra Nevada. The initial investigation followed an outbreak of coccidioidomycosis among UCLA anthropology students, and the authors recovered *C. immitis* from the midden but not from 80 soil samples nor from more than 400 rodents collected within 4.5 miles of the midden.[9] A reinvestigation of the midden published in 1974 reported the same result, that is, *C. immitis* could be recovered from the midden but not from adjacent soils.[10] As a result of these investigations, a new hypothesis emerged, that the reservoir for *Coccidioides* species was soil and that the distribution and prevalence of the fungus could be determined by examining aspects of the soil and climate. Many studies of soil and climate as they affect *Coccidioides* species have been conducted from the 1950s until today with a common theme that, in areas of sandy soil, changes in the incidence of coccidioidomycosis follow, by a year or more, shifts in the amount of precipitation.[11–15]

The small mammal reservoir hypothesis was resuscitated when comparison of the genomes of *Coccidioides* species with other Ascomycota showed an expansion within the genomes of *Coccidioides* species of genes coding for enzymes that allow the fungus to digest animal protein and the loss of some genes coding for enzymes that allow other Ascomycota to digest plant cell walls.[16] The inference of these results is that *Coccidioides* species evolved to feed on animal protein, a change that selected for the retention of duplications of genes associated with feeding on animals and eliminated selection to retain genes involved in feeding on plants. This is not to say that *Coccidioides* species cannot grow on dead parts of plants, which has been observed in nature.[17] However, nutritional experiments with a close relative of *Coccidioides* species that experienced the same gene family contractions and expansions, *Uncinocarpus reesii*, demonstrated a preference for protein over polysaccharide.[18] The most likely interpretation is that a common ancestor of *Coccidioides*, *Uncinocarpus*, and related species evolved to eat animals and then abandoned the ancestral state of eating plants. Additionally, because we know that *Coccidioides* is not transmissible among living mammals, the spherule would be an evolutionary dead

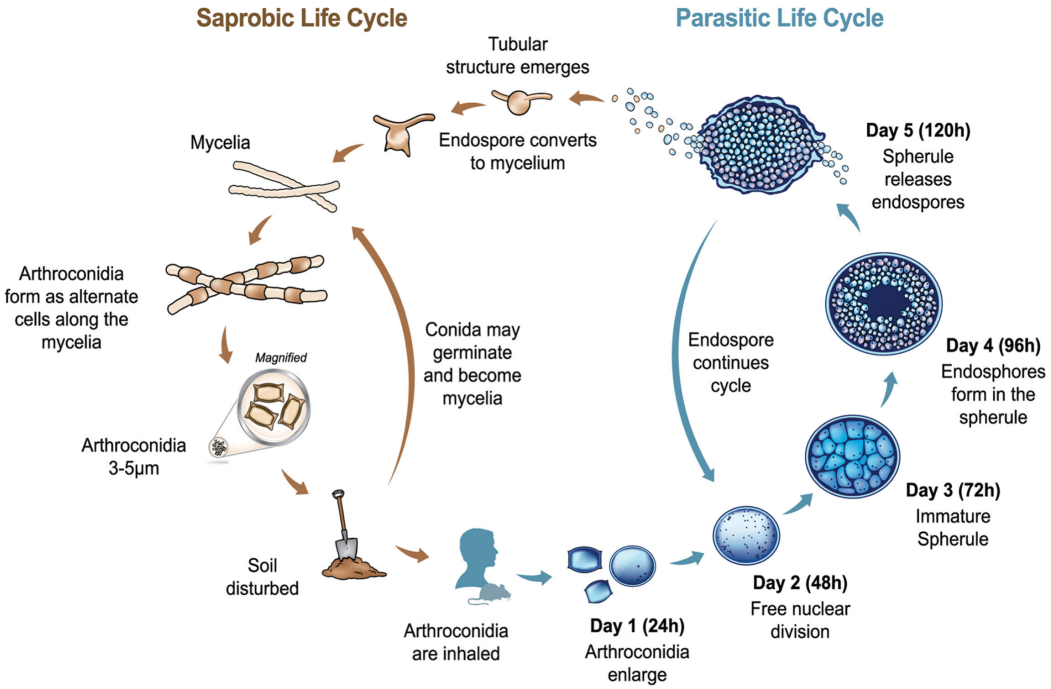

Figure 2. Modern, medical life cycle of *Coccidioides* species. In the environment, *Coccidioides* is thought to grow as a septate mycelium, and every other cell will mature into arthroconidia. Arthroconidia of both *Coccidioides immitis and C. posadasii* shift into a parasitic spherule cycle in a susceptible host. It has been observed that spherules become mature in 5–10 days, although exact timing is dependent on complex host-pathogen interactions. The mature spherule is an encasement for uninucleate endospores, which are released if rupture occurs. The 1–3 μM diameter endospores can remain in the lung tissue, or disseminate to multiple body sites including liver, spleen, lymph nodes, meninges, joints, and bones. Mycelia can be cultured easily from these infected tissues or fluids. Image[39] used under Creative Commons License. This Figure is reproduced in color in the online version of *Medical Mycology*.

end if the fungus could not revert to the mycelial phase to produce arthroconidia and escape the infected host to find a new, naive host.[2]

The resurrected endozoan, small-mammal-reservoir hypothesis posits that *Coccidioides* infects a large enough percentage of small mammals to maintain its presence in the environment,[5] that after infection the fungus persists in living mammals as spherules in granulomas,[4] that the living fungal spherules convert to mycelium when the host dies (due either to disseminated coccidioidomycosis or other natural causes), and that the mycelium then produces abundant arthroconidia that spread the fungus to new hosts.[19] Regarding the sporulation of *Coccidioides* spp. in the carcasses of dead mammals, it has been shown that soil in the endemic area of C. *immitis* that failed to produce cultures of C. *immitis* for 3 years became positive 5 months after the burial of mice that had been experimentally infected with the fungus and remained positive for each of the subsequent six years of investigation.[20] It is also known that predation or scavenging of infected animals by birds or mammals prevents *Coccidioides* from exploiting the dead prey.[21] Therefore, it would be important to know the proportion of small mammals that die from causes other than predation and the fate of their carcasses. Research in this area is scant, but there are estimates that predation accounts for a small proportion of deaths,[22] and it has been shown, experimentally, that dead, native rodents added to burrows are walled off in small burial chambers and not removed.[23] If the naturally buried rodents were infected with *Coccidioides*, it seems safe to assume that spores would be produced, as has been shown with experimentally buried mice.[24] Despite this focus on small mammals, one cannot preclude a role in the *Coccidioides* life cycle of larger desert mammals (e.g., coyotes), given the presence of coccidioidomycosis in canids.[24]

The effect of soil and climate can be reevaluated in light of the endozoan hypothesis. Three themes are common to studies of the effect of climate on the presence of *Coccidioides*: high temperatures, low precipitation, and an increase in the incidence of coccidioidomycosis 1 to 2 years following a change in precipitation.[11–15] These climate parameters certainly can have a direct effect on the growth of *Coccidioides* species, but they also would have an indirect effect on *Coccidioides* species through effects on the abundance of plants and, in turn, the abundance of herbivorous animals (Fig. 3). Remembering that *Coccidioides* species have evolved to digest animal protein, and that rodent species known to harbor *Coccidioides* species are herbivores, the climate affecting plant growth must have an effect, appropriately delayed, on the abundance of *Coccidioides*.

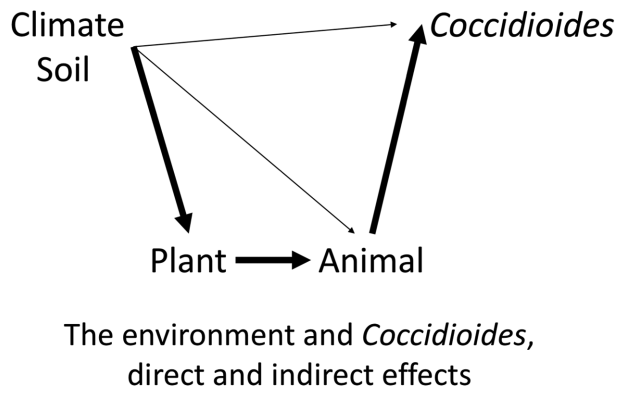

Figure 3. Effects of the physical environment on plants, animals and *Coccidioides* species. Environmental parameters of temperature, moisture and soil composition have a direct effect on *Coccidioides* fungi but also have indirect effects on the growth of plants that feed the small mammals and on the small mammals that provide nutrition for the fungi. Given that *Coccidioides* species evolved to live on animal protein and then lost genes coding for enzymes used to digest plant cell walls, the indirect effects of the physical environment on plants and animals are likely to be greater than any direct effects on the fungi.

For example, increased precipitation in the present year would lead to increased plant growth that season. Increased availability of plants would allow the small mammal population to increase. Increased availability of small mammals would allow the *Coccidioides* population to increase. Given the high mortality of small mammals even in periods of plant abundance, particularly in their first year of life, in the year or years following unusual precipitation, there would be an increase in the amount of *Coccidioides* infective propagules, the arthroconidia.

Similarly, correlation between soil parameters and the presence of *Coccidioides* species could be due to soil parameters affecting the growth of plants and the presence of small mammals as well as parameters that directly affect the fungi. For example, the key parameter of soil moisture impacts the growth of plants, and the almost universally reported parameter of soil consistency, very fine sand and silt,[25] affects the ability of small mammals to construct burrows. By any hypotheses concerning the growth and sporulation of *Coccidioides* species, the climate parameter of high velocity wind is associated with increased dispersal of arthroconidia,[26] provided that there are viable propagules in the soil. Of course, disturbance of soil by humans also aids arthrospore dispersal, as demonstrated by outbreaks associated with human displacement of soil.[27–29]

We propose that models seeking to explain the incidence of coccidioidomycosis by accounting for physical environmental parameters will be improved by also accounting for biological environmental parameters,[30] such as plant and small mammal abundance. A careful example of this approach just appeared, demonstrating that California's recent, severe drought in a *C. immitis* hyperendemic region caused dramatic effects on plant and rodent populations.[31] Doubtless, a key parameter will be the percentage of infection in populations of small mammals. These data would also be useful for estimating the risk of contracting coccidioidomycosis, again as noted by Chester Emmons in the early 1940s.[32] Of course, these same data would be useful in attempting to disprove the endozoan, small-mammal reservoir hypothesis presented here.

The puzzle of the research that quashed the small-rodent reservoir remains unsolved, that is, why would *Coccidioides* be recovered from a Native American midden but not from soil or animals surrounding the midden. One possible solution is that middens contain sufficient nutrients consisting of bone, carcasses, and excrement that allow organisms to persist in the midden long after its creation. This scenario has been explored with plants but not fungi.[33] A final puzzle concerns the absence of *Coccidioides* species from mountain forests adjacent to dry areas of endemicity. These wetter areas support the same types of small mammals as do the dry ones and more of them, but coccidioidomycosis is not associated with these areas. Perhaps the wetter conditions allow bacteria to dominate the decay of small mammal carcasses, as well as to decay organic contents of middens, thereby depriving *Coccidioides* of the resource that serves it so well in dryer zones. There is also the possibility that adaptation to desert environments preceded evolution of the endozoan habit. Perhaps there are "near phylogenetic neighbors" of *Coccidioides* that do not cause clinical disease in humans and remain to be discovered in dry environments. The combination of high temperatures and harsh, nutrient-poor soil conditions might naturally drive organisms to a stronger association with nutrient rich and hydrated mammals to live and reproduce in harsh desert conditions.

As noted above, hypotheses must be tested. The most direct, prospective experiment is not possible, that is, to extirpate small mammals from a large, endemic area and then, over the following decades, observe the rate of human coccidioidomycosis in that area relative to neighboring regions. However, similar experiments are routinely conducted when wild lands are brought into cultivation with the result that *Coccidioides* is, retrospectively, reported to be excluded.[17] However, in the agricultural soils not only have the wild rodents been excluded by cultivation, but water has been added through irrigation, confounding the interpretation. An attractive alternative, mentioned above, would be to determine the percentage of small mammals harboring *Coccidioides* to see if fluctuations in incidence correlate with the frequency of human infections. Skin testing using antigens derived from hyphae[34] or spherules[35] formed the basis of the foundational studies of the incidence of *Coccidioides* infections in humans. Skin testing detects the cellular based immunological response. An alternative to skin testing, serology, which detects the antibody response, has been used to detect *Coccidioides* in small mammals,[36] but there are two drawbacks to its use: antibodies to *Coccidioides* do not persist more than a year or two so one can miss quiescent *Coccidioides* in old granulomas, and the animals must be sacrificed because of the amount of blood

required for the test. The first drawback could be overcome through skin testing of small mammals using a newly available antigen present in spherules,[37] but the lengthy period, 36 hours, needed to detect swelling of rodent paws may make this approach impractical. The second drawback could be overcome through the use of tests for antibodies that require less blood, which are newly available for dogs and could be adapted for use in small rodents.[38] New technologies to test T-cell proliferation in small mammals, such as an ELISPOT, which should be able to test a variety of host species, might also be useful if the test could be modified to work with very small amounts of blood. Finally, we recognize that there are many unanswered questions that remain, and we anticipate a great deal of progress in the next few years to address the important environmental reservoir of *Coccidioides*.

Acknowledgments

This work was supported by the University of California Office of the President Multi-campus, Research Program, Grant MRP-17-454959 to JWT and the Arizona Biomedical Research Commission Grant ADHS-16-162415 to BMB.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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