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Molecular detection of *Coccidioides* spp. from environmental samples in Baja California: linking Valley Fever to soil and climate conditions

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ABSTRACT

Coccidioidomycosis is an important human fungal infection of American deserts and nearby semi-arid regions with highly endemic areas distributed along the United States-Mexico border. Despite the increasing incidence in the last 20 yr, reports of positive isolations of the causal agent, *Coccidioides* spp. from environmental samples have been scarce. To resolve this paradox, it is extremely important to first identify the fundamental ecological niche of this fungus. Soil samples ($n = 90$) including those from heteromyids' active burrows, latrines and other mammals' dens were collected using an oriented sampling method from areas of Baja California, Mexico previously predicted as putative endemic "hotspots". The total genomic DNA obtained from the collected samples was subjected to a nested PCR followed by a diagnostic PCR designed to amplify the internal transcribed spacer (ITS) 2 region of *Coccidioides* spp. From the 42 amplicons obtained and sequenced (37 from Valle de las Palmas (VDP) and five from San Jose de la Zorra (SJZ)), 32 were confirmed to belong to *Coccidioides* spp. No *Coccidioides* spp. were found in soils collected in Ensenada.

VDP and SJZ have different soil characteristics but share a Mediterranean climate having less than 250 mm of precipitation per year, as well as a dry period of at least 6 months. The development of *Coccidioides spp.* is probably related to the structure of the microbial population adapted to these conditions in the semi-arid-mediterranean ecotone.

Introduction

Coccidioidomycosis, also known as Valley Fever, is an endemic fungal disease caused by the dimorphic Ascomycetes *Coccidioides* spp. *Coccidioides immitis* is found in San Joaquin Valley in Southern California in one of the most important endemic areas in the United States, whereas *Coccidioides posadasii* is localized in Southern Arizona and in South America, where cases have been found mostly in Argentina, Venezuela and Brazil. In Mexico, most of the cases have been reported in the states of Sonora,

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Nuevo Leon, Coahuila and Baja California. Specifically, in northern Baja California, most reported cases are of *C. posadasii* with only a few reported cases of *C. immitis* (Laniado-Laborín *et al.* 1991; Fisher *et al.* 2001; Castañón-Olivares *et al.* 2007). This type of geographical distribution of the disease was established over 40 yr ago according to the epidemiological data at the time (Maddy & Coccozza 1964; reviewed by Baptista-Rosas & Riquelme 2007). Recent epidemiological research has reported incidence rates of 150 cases/100 000 population in Kern county, California (Vugia *et al.* 2009), with outbreaks of more than 500 cases/100 000 population (Zender & Talamantes 2006; Flaherman *et al.* 2008). Similarly, there are reports of 159 cases/100 000 population in Maricopa County, Arizona (Komatsu *et al.* 2003). Altogether there are estimates of more than 200 000 cases annually in the United States alone (Buckley 2008), which represent about a six-fold increase since 1995. The incidence of the disease in Mexico is unknown because it is not mandatory to officially report and register the cases. However, ongoing studies have identified highly populated areas along the United States-Mexico border endemic for coccidioidomycosis (Ampel *et al.* 1998; Hector & Laniado-Laborin 2005; Flaherman *et al.* 2008). The highly endemic areas in southern California and Arizona share biogeographical and bioclimatic characteristics with some areas of Baja California previously identified as potential hotspots for the presence of the fungus (Baptista-Rosas *et al.* 2007). These similarities suggest that the disease may have a comparable epidemiological distribution across the border.

Coccidioidomycosis cannot be transmitted from person to person, but acquired by inhalation of the arthrospores from the environment where the fungus inhabits; therefore the fungus should be isolated from endemic areas (Ajello *et al.* 1965; Ajello 1967; Lacy & Swatek 1974). However, the scarce environmental evidence for *Coccidioides* spp. seems to be in disagreement with the high incidence rates obtained for the disease. Only a few positive isolations from environmental samplings have been obtained in highly endemic areas in the United States (Stewart & Meyer 1932; Emmons 1942; Maddy 1965). For instance, only 13 soil isolations were reported in California and one in Arizona after extensive sampling using mice intraperitoneal inoculation and culture isolation (Swatek 1970). More recently, only four positive isolations out of 720 soil samples were obtained in California combining microbiological selective isolation techniques and PCR diagnosis (Greene *et al.* 2000) and no positive detections were obtained using a semi-selective method and direct genomic DNA isolation followed by PCR from 150 soil samples in Arizona (Tabor *et al.* 2002). In other studies, 62 environmental positive isolates from 11 sites in the Tucson area were obtained by intraperitoneal inoculation of soil extracts into female BALB/c mice (Mandel *et al.* 2007). In Mexico, by using this last methodology only two positive records have been reported; one from Hermosillo, Sonora (Sotomayor *et al.* 1960), and another one from Valle de las Palmas (VDP), 17 miles south of Tecate, Baja California (Cairns *et al.* 2000).

We suspect that the low number of positive isolations in previous studies could be due in part to a non-directed sampling strategy given the poor characterization of *Coccidioides* ecological niche and its role in the structural dynamics of the microbiological community of soil deserts, rather than the methods used to process the collected samples. Even though the requirements and conditions for *Coccidioides* growth in the laboratory have been well characterized, the total range of suitable environmental conditions for its development in the natural habitat has not been well defined (Barker *et al.* 2007; Fisher *et al.* 2007). Thus suggesting that efforts should be invested towards the identification of *Coccidioides* spp. ecological's niche (Maddy 1957, 1958; Fisher *et al.* 2007). Furthermore, the climatic characteristics of the region are also important factors for *Coccidioides* spp. development (Maddy & Coccozza 1964; Comrie 2005). In the last two decades increasing rates of prevalence and incidence of this disease have been directly correlated with rainfall in usually dry regions in Arizona (Park *et al.* 2005; Zender & Talamantes 2006). The available evidence shows that outbreaks of the disease appear a year or two after an abnormal weather-related rainfall increase, following a period of prolonged drought (Comrie & Glueck 2007; Baptista-Rosas *et al.* 2010).

The current state of the disease and its correlation to the arid and semi-arid climate conditions of northern Mexico have not been studied with the attention they deserve. Bearing all this in mind, we have conducted an oriented environmental sampling strategy in the most likely fundamental ecological niche for *Coccidioides* in two semi-arid areas of Baja California previously predicted as endemic hotspots (Baptista-Rosas *et al.* 2007) and used nested PCR followed by diagnostic PCR to explore the presence of *Coccidioides* spp. These studies are part of a long-term multidisciplinary project aimed to study the impact of global climate changes in the distribution of the Valley Fever fungus in Baja California.

Methods

Soil sampling strategies

Using available coccidioidomycosis epidemiological information and data on ecological niche modelling obtained by combining the Genetic Algorithm for Rule Set Production (GARP) and Geographical Information Systems (Baptista-Rosas *et al.* 2007), we designed two sampling polygons in predicted endemic areas of Baja California, Mexico: San José de la Zorra (SJZ), 38 km north of the Ensenada; and Valle de las Palmas (VDP), 17 km south of Tecate (Fig 1). As reference, we collected soil samples from Ensenada (Fig 1). We conducted an oriented soil sampling strategy (at least 10 samples per polygon) within 16 km^2 polygons set around areas with evidence of small mammals' activity (Fig 6). For VDP and SJZ, soil samples from heteromyids' active burrows, latrines and other mammals' dens were included. Soil samples from about 10 cm under the surface were collected also for all locations. For each sampling site, 20 g of soil were collected. The shovel was cleaned with hydroxide chloride in between samples. A clean plastic disposable spoon was used for each sample, which was collected into a sterile 90 ml specimen collection cup.

Sampling dates were selected according to a bioclimatic analysis defined by the relationship between the number of reported cases of the disease and the monthly mean precipitation from 1971 to 1995, where most of the reported cases of the disease were registered mainly in the driest months of the year after seasonal rains (Baptista-Rosas *et al.* 2010). Therefore