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Oral Presentations

1

Disease Triangle Dynamics of Coccidioidomycosis in Northern Arizona; a One Health Approach

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Abstract

Coccidioides species are the etiological agent of the disease coccidioidomycosis (a.k.a Valley fever). Human disease ranges from asymptomatic to chronic pneumonia, and in severe cases dissemination beyond the respiratory system. If diagnosis is delayed or missed the risk of severe disease increases. Nationally, the incidence of newly diagnosed cases is increasing and Arizona has some of the highest prevalence of diagnosed infections reported each year, which predominantly occur in southern Arizona. In moderately endemic regions such as northern Arizona, the risk of acquiring disease is lower than in southern Arizona, but still prevalent and on the rise. The presumption of reduced risk in northern Arizona, can lead to less awareness of the disease in the public or health professionals. One approach to understanding the risk of an infectious disease is a concept called the disease triangle. This uses a holistic approach where the intersection of pathogen, host and environment are considered. We sought to investigate the disease triangle dynamics of Valley fever in northern Arizona. First, we considered host factors by inspecting the number of reported cases over time, followed by examining Valley fever cases treated at the regional hospital in northern Arizona. We then turn our attention to the pathogen and used genetics to compare northern Arizona clinical isolates to other Arizona clinical strains. Lastly, we conducted an environmental survey of northern Arizona soils for evidence of *Coccidioides* using two real time qPCR assays. We found that cases of Valley fever are increasing in the northern Arizona region and individuals are experiencing severe disease requiring hospitalization. The local clinical isolates are genetically related to southern Arizona populations, demonstrating infection may be acquired during travel to these highly endemic regions. However, we did detect DNA from *Coccidioides* in all of the northern Arizona counties, indicating the presence of a novel species or close relative of *Coccidioides* in the area. Together our data provide the first insight into the local Valley fever disease triangle. Until preventing the disease is a viable option, we expect that cases will continue to increase in the area. This perspective indicates increased awareness and screening for the disease is crucial to limit severe disease in the region.

2

Δcps1 LIVE AVIRULENT VACCINE PROTECTS DOGS AGAINST EXPERIMENTALLY INDUCED PULMONARY COCCIDIOIDOMYCOSIS

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Abstract

Introduction: Coccidioidomycosis is a problem that both physicians and veterinarians in endemic regions, especially Arizona and California in the U.S., must address on a daily basis. Both humans and dogs would greatly benefit from a vaccine to prevent this fungal disease. A live, avirulent vaccine candidate, *Δcps1*, was tested for tolerability, safety, and efficacy for prevention of pulmonary coccidioidomycosis in a canine challenge model.

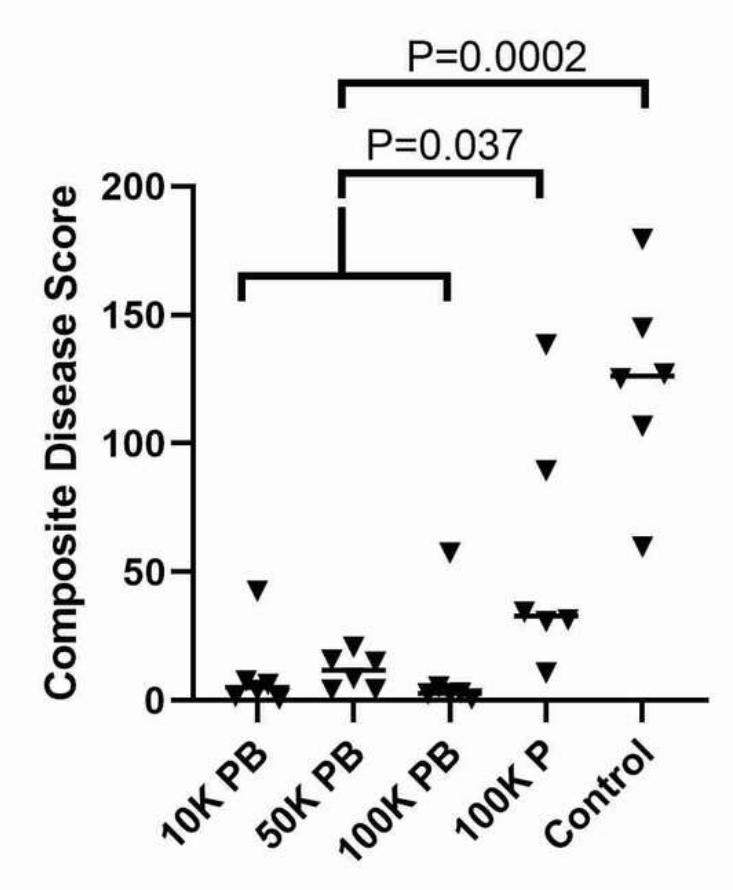
Methods: Thirty-four male and female beagle/beagle mix dogs, 1-2 years old, were randomized and vaccinated subcutaneously. Seven received 100K arthroconidia only once (prime only [P]), 7 others received 10K, 50K, or 100K live *Δcps1* arthroconidia and again 28 days later (prime/boost [PB]), and 6 received saline injections as a control [C]. Blood was collected for serology before vaccination and every two weeks through day 42 post-vaccination. A dog from each *Δcps1* vaccination group was euthanized 2 weeks after the second administration to culture injection sites and draining lymph nodes for residual *Δcps1*. The remaining 30 dogs were infected 4-6 weeks after vaccination by intratracheal nebulization of 10,000 arthroconidia of *C. posadasii* strain Silveira. Investigators were blinded to the treatment. Dogs were monitored daily and examined weekly; radiographs, CBC, and serum for chemistries and serology were collected every 2 weeks, including on the day of euthanasia, 56 days post-infection. At necropsy, lung lobes were separated, weighed, and homogenized to determine lung fungal burden. One tracheobronchial lymph node was also cultured. Samples of ~1 cm^3 collected from each lung lobe, the liver, spleen, kidney, and a thoracic lymph node were fixed for histopathology (H&E stain). Board-certified veterinarians blinded to the treatment groups evaluated and scored the radiographs and histopathology. Lung fungal burdens were compared by Kruskal-Wallis. A composite score was determined for each dog from the $\log_{10}$ lung and lymph node fungal burdens, reciprocal of serum immunodiffusion titer, total radiology score, total histopathology score, lung nodule score, lymph node score, scores for neutrophilia, monocytosis, hyperglobulinemia, hypoalbuminemia, and reciprocal of albumin-globulin ratio (A/G ratio). Composite scores were assessed by Kruskal-Wallis.

Results: Vaccine injection-site reactions were transient and there were no systemic effects in dogs given one or two subcutaneous vaccinations of viable *Δcps1* arthroconidia. In the 100K PB dog, a colony grew

from the residual homogenate (900 µL) of the primary injection site tissue; *Δcps1* was not detected in any other injection sites or the draining lymph nodes. Antibody responses from vaccination were rarely detected. Infection with virulent strain Silveira produced no clinical disease and minimal pathology in dogs vaccinated twice. There was no difference in the lung fungal burdens (P>0.99) or composite disease scores (P=0.2306) of the PB groups, so these were combined for statistical analysis. Regardless of the dose, PB significantly reduced lung fungal burdens compared to prime only or saline (P<0.001, both comparisons). The composite disease score of the PB dogs (mean score 10.6) was significantly lower than prime only (mean score 54.8, P=0.037) or saline (mean score 121.2, P=0.0002)(**Fig. 1**). Although prime vaccination only resulted in modest mitigation, the quantitative measures of disease were not significantly different from the control dogs.

Conclusion: The live vaccine was well-tolerated in the dogs with minimal persistence of vaccine organisms and a high level of protection. These results drive the *Δcps1* vaccine toward a commercial veterinary vaccine and support continued development of this vaccine to prevent coccidioidomycosis in humans.

Fig. 1. Composite disease scores by vaccination group. The mean disease scores were similarly low (P=0.2306) in the three groups that received both prime and booster vaccinations so they were combined for further analysis. Two vaccinations resulted in significantly lower disease scores than one vaccination or no vaccinations. Bar = median score. (Statistical analysis – Kruskal-Wallis)



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CHARACTERIZING *IN VITRO* AND *IN VIVO* BIOMARKERS FOR A VALLEY FEVER BREATH TEST

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Abstract

INTRODUCTION: There is a critical need for sensitive, non-invasive diagnostics for detecting Valley fever lung infections; therefore, we are working toward identifying volatile organic compound (VOC) biomarkers of *Coccidioides immitis* and *C. posadasii* infections via metabolomics analyses of *in vitro* cultures, murine model lung infections, and lung specimens from humans with Valley fever. Herein, we present recent data on the VOC profiles of *C. immitis* and *C. posadasii* grown *in vitro*, and the VOC profiles of bronchoalveolar lavage fluid (BALF) samples from mouse model lung infections with each species.

METHODS: *In vitro:* Six strains each of *C. immitis* (three SJV and three SDMX strains) and *C. posadasii* (three AZ and three TX/MEX/SA strains) were cultured in triplicate for 96h in RPMI 1640 with 10% FBS media at 39ºC in 10% CO_2 to induce spherule formation, and normoxia at 30ºC for mycelial formation, yielding 72 cultures. The spent media were filter-sterilized for VOCs analyses by headspace solid phase microextraction (HS-SPME) and two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS). *Murine model:* The protocol for animal infection (16-009) was approved by the Institutional Animal Care and Use Committee at Northern Arizona University, in accordance with Animal Welfare Assurance A3908-01 from the US Department of Health and Human Services. Three cohorts of female C57BL/6 mice were infected by intranasal inoculation with *C. immitis* RS (n=6), *C. posadasii* Silveira (n=6), or vehicle control (n = 4). A dose of 100 conidia in 30 µl sterile PBS was used for infection. The mice were allowed to develop symptoms for 10 days and then euthanized. Tracheal intubation followed by PBS washing recovered approximately 2 mL of BALF for VOCs analysis by HS-SPME-GC×GC-TOFMS and for cytokine analysis by a mouse magnetic 26-Plex ProcartaPlex™ panel. Mouse spleen and brain were homogenized in 1 mL of sterile PBS followed by culture of 10-fold dilutions of each tissue on 2X GYE agar to quantify fungal dissemination. *Statistical analysis:* Hierarchical clustering analysis (HCA) and principal component analysis (PCA) was performed on the individual data sets. Pearson correlation was calculated for the VOCs and cytokines in the murine model data set.

RESULTS: We detected a total of 353 VOCs that were at least two-fold more abundant in a *Coccidioides* culture versus medium controls. The total number of VOCs produced by *C. immitis* versus *C. posadasii* were similar, at 309 versus 291, respectively. Controlling for lifecycle (spherule vs. mycelia), we did not observe any segregation between species by their VOCs via PCA. Within each species, the two lifecycles are quite divergent in their volatilomes. We found the volatilome of *Coccidioides* is more dependent on lifecycle than species.

Preliminary analyses of the BALF from *Coccidioides* infected mice indicates that VOCs are correlated to cytokine production (Figure 1). We did not observe any separation between the *C. immitis* and *C. posadasii* infected mice by their BALF VOCs via PCA; rather, mice separate based on their individual level of infection, indicated by their immune response and the degree of fungal dissemination.

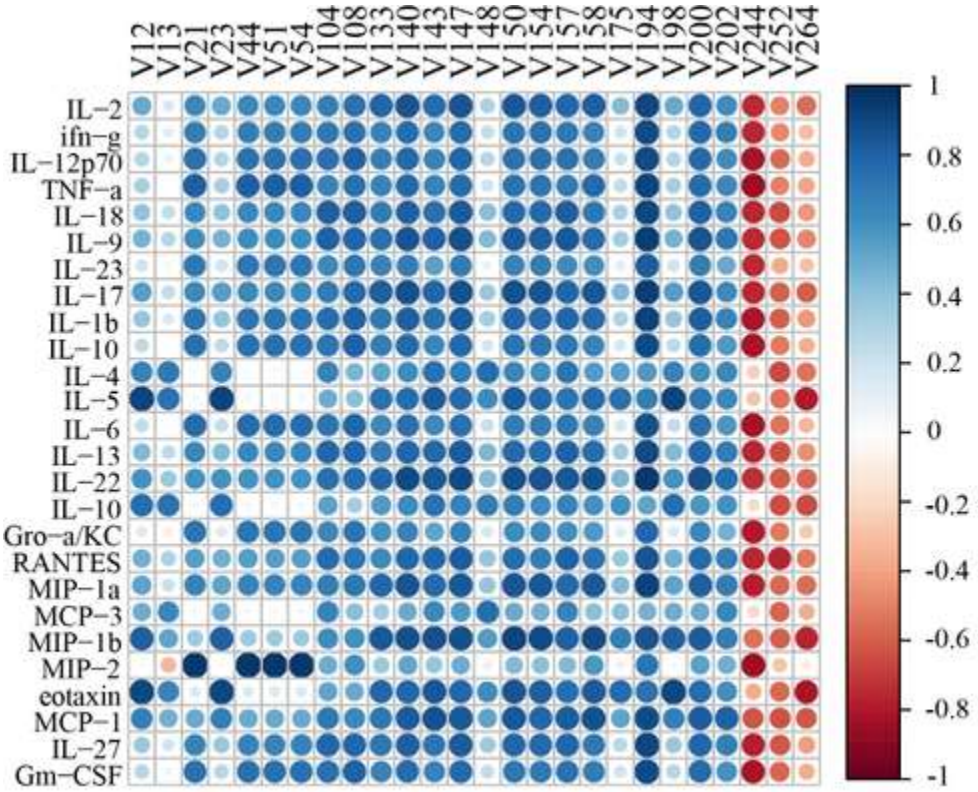

Figure 1. Pearson correlation plot of the abundance of 26 VOCs and cytokines in 12 inoculated and 4 control mice. The 26 VOCs are significantly ($p < 0.01$) positively (> 0.7) or negatively (<-0.7) correlated to cytokine production. Darker color and larger size of the markers indicate stronger correlation; blue = positive correlation, red = negative correlation

CONCLUSIONS: Our pilot data indicate that *Coccidioides* spp. produce VOCs that may yield biomarkers for a Valley fever breath test. The next steps of this work will be to collect the VOCs from lung specimens (lavage and sputum) from persons with community-acquired pneumonia, and determine which of the *in vitro* and murine model Valley fever biomarkers can differentiate between bacterial and fungal etiologies of disease.

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CONTRIBUTION OF BIOLOGIC RESPONSE MODIFIERS TO THE RISK OF COCCIDIOIDOMYCOSIS SEVERITY

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Abstract

Introduction. A small percentage of coccidioidomycosis (CM) in otherwise healthy individuals results in life-threatening respiratory illness or disseminated CM (DCM). The risk rises as much as 150-fold in immunosuppressed patients. An ongoing clinical question is the safety of using biologic response modifiers (BRMs) in CM-endemic regions. Our team sought to determine the risk BRMs pose to patients with autoimmune (AI) disease in the Tucson and Phoenix areas.

Methods. We conducted a retrospective study to review Banner Health electronic medical records (EMR) of patients ≥18 years old, with CM and AI diagnoses from 10/01/2017 to 12/31/2019. Seven physicians and medical students extracted detailed information including demographics, length of Arizona residency, clinical presentations, AI diagnoses, disease severity, CM confirmatory test results and BRM treatment. Categorical variables were summarized as counts (percentage) and continuous variables were summarized as medians. Differences in proportion for categorical data were analyzed by the Fisher exact test and continuous variables were analyzed by Student *t* test.

Results. A total of 7,249 AI patients were identified via the EMR. 944 of 7,249 records had ICD-10 codes for both AI and CM. Of these, 615 (65%) had CM confirmed by serology, pathology, or culture. The median age was 52 years, 57% were male, and 73% were Caucasians. Of 615 CM patients, 67% were primary pulmonary CM (PCM) and 11% were DCM. Among DCM patients, 31% were of African ancestry ($p<0.001$). CM-positive patients had lived in Arizona a median of 10 years whereas median endemic residence of patients without CM was 22 years ($p=0.04$). Most AI patients had rheumatological disease. The three most commonly used BRMs were adalimumab, etanercept and infliximab. 6% of PCM and 1.8% of the DCM patients were on BRM when diagnosed. For patients on BRM and steroids this percentage was 16% and 15.8% for PCM and DCM, respectively.

Conclusion. Our study indicates that 65% of the patients with CM ICD-10 codes had a laboratory-confirmed diagnosis. PCM was not generally an indication for BRM discontinuation. Confirmed DCM, however, led to BRM discontinuation in the great majority of cases. Addition of steroids to BRM treatment increased both PCM and DCM risk. That most CM developed in patients with less endemic exposure suggests new infections are more frequent than reactivation.

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EFFICACY OF NIKKOMYCIN Z IN MURINE CENTRAL NERVOUS SYSTEM COCCIDIOIDOMYCOSIS, MODELING SUSTAINED RELEASE DOSING

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Abstract

Introduction Meningitis is the most feared complication of coccidioidomycosis. Nikkomycin Z (nikZ) is a chitin synthase inhibitor, shown active against different manifestations of coccidioidomycosis. A concern with possible future nikZ therapy is its short half-life. This has resulted in the use of multiple dose/day regimens in animal experiments. Here we examine the possibility that an extended release form, which would provide steady state blood levels for a therapy duration, while relinquishing bolus pulses, could prove efficacious. Extended release would enhance convenience, and adherence, for patients, and possibly also improve efficacy. We simulated a possible extended release form by providing the drug *ad libitum* in drinking water to infected mice.

Methods. *Coccidioides posadasii* (82 arthroconidia) was injected intracerebrally into groups of 9-10 CD-1 female mice. Treatment began on day 3, for 12 days. Fluconazole was given 100 mg/kg QD by gavage, and designed doses of nikZ 30, 100 or 300 mg/kg/day given in drinking water, and intake monitored. Post treatment, mice were observed 30 days, then survivors euthanized, brain CFU quantitated, and CFU in other organs (lung, liver, spleen, kidney) assessed semiquantitatively.

Results MIC and MFC for fluconazole were 6.25 and >100 mcg/ml, nikZ 2.5 and 2.5 mcg/ml. Stability of nikZ in drinking water was confirmed.

GROUPS	**Control**	**Fluconazole**	**nikZ 30**	**nikZ 100**	**nikZ 300**
Survival %	11	50	70	90	100
Survival p vs. control	-	0.01	0.005	0.001	<0.0001
% brain sterilization, survivors	0%	20%	86%	89%	80%
p vs. control	-	Not significant	0.01	0.001	0.001
Sterilization all organs, survivors	0%	20%	86%	89%	80%

Re survival, nikZ 300 was superior to fluconazole, p= 0.01. Re brain sterilization, nikZ 100 and 300 were superior to fluconazole, p= 0.01. Survivors without brain CFU had no other organs infected. The only 2 survivors, in all nikZ mice, with other organ infection, had only 1-2 CFU/organ; CFU in fluconazole-treated trended higher, and for three organs nikZ 100 or 300 was p ≤0.03 more efficacious.

All mice decreased drinking after infection, causing nikZ mice to ingest less than their desired dose in early therapy; despite this, by day 3 of treatment they had recovered sufficiently to resume pre-infection drinking rates and thus approximated their designed drug intakes. Thus, when sickest, even

less than their designed dose was sufficient to enable recovery. Overall, the nikZ 30, 100, 300 groups ingested 82%, 97% and 99%, respectively, of their total designed dose.

Conclusion Efficacy in this study supports the development of a sustained release form of the drug in man. Decreased intake by sick mice would not be expected to be a factor in therapy of humans, receiving the drug via an extended release pill or by continuous intravenous infusion, where intake would be more assured. Either preparation could also maintain drug delivery even during sleep periods. In two prior studies of murine coccidioidal meningitis with similar inocula, survival with 100 mg/kg/day nikZ divided into oral twice daily administration produced 60% survival, inferior to our results. That suggests, in addition to the convenience of sustained release, sustained release may be a superior therapeutic modality.

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Update on Coccidioidomycosis Epidemiology Amidst a Respiratory Disease Pandemic

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Abstract

Introduction: Amid one of the worst respiratory disease pandemics in a century, coccidioidomycosis remains a public health threat. Fever, cough, and persistent fatigue are hallmarks not only of COVID-19 but are also of coccidioidomycosis, potentially resulting in missed diagnoses. As the pandemic unfolded in 2020, its impact on diagnosis and reporting of coccidioidomycosis was unknown. We summarize recent trends in U.S. coccidioidomycosis epidemiology and public health activities for this neglected but important disease.

Methods: We examined public health surveillance data, published literature, and reports from the field to summarize coccidioidomycosis epidemiologic trends in the United States. We also summarized recent public health activities.

Results: Annual reported U.S. coccidioidomycosis cases remain well above their recent nadir in 2014 and remained elevated in 2020 despite the pandemic. In 2018, about 15,000 cases were reported to CDC. Cases increased substantially in 2019 to >19,000, with Arizona reporting ~10,000 cases and California ~9,000 cases. In 2020 preliminary state surveillance data, Arizona coccidioidomycosis cases increased to ~11,500, while cases declined slightly in California to ~6,500 compared to the previous year. Monthly reported cases declined in March–May 2020 but rebounded as the year progressed. We will discuss several data sources to examine changes in diagnostic practices during the pandemic and coinfections.

Understanding the geographic distribution of coccidioidomycosis is helpful in targeting prevention and control measures. A new CDC public-facing map, based on data from public health surveillance, outbreaks, and environmental detection, aims to better convey the broad geographic regions at risk, as well as uncertainty for many areas; and a recent paper aims to model the soil habitat of the fungus using a range of soil and environmental inputs in national soil databases. We are also seeking to strengthen public health surveillance, which has been strained by the pandemic, as it is essential to track the impacts of intensifying climate change on the geography and incidence of this disease. Improved surveillance can also inform efforts to improve coccidioidomycosis prevention and clinical care. We will also discuss efforts to update the national public health case definition for coccidioidomycosis, describe a draft diagnostic algorithm to aid front-line clinicians less familiar with this illness, and briefly present the preliminary findings of *Coccidioides* infections among patients with acute respiratory illness.

Conclusions: The COVID-19 pandemic has further accented the urgency for public health institutions to cohesively collect and analyze coccidioidomycosis data in multifaceted ways. We look forward to working with the Coccidioidomycosis Study Group to enhance public health actions to prevent disease and death from *Coccidioides* infection.

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Development of a Coccidioidomycosis Diagnostic Algorithm for Primary Care Providers in the United States

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Abstract

Introduction:

Coccidioidomycosis is often misdiagnosed and is frequently treated empirically as bacterial community-acquired pneumonia. Accordingly, nationwide surveys have shown low public awareness and low levels of testing for coccidioidomycosis by primary care providers, who sometimes express uncertainty about which diagnostic tests to order and how to interpret them, given the many options available.

Methods:

We reviewed the literature on the performance characteristics of currently available diagnostic tests for *Coccidioides* infections, taking into account characteristics such as sensitivity, specificity, test availability, turnaround time, and cost. We synthesized this information into a draft coccidioidomycosis diagnostic algorithm, with an intended target audience of primary care providers in the United States.

Results:

The draft coccidioidomycosis diagnostic algorithm recommends *Coccidioides* enzyme immunoassay (EIA) antibody testing for immunoglobulin M and immunoglobulin G as the first-line test, with lateral flow assay as an acceptable alternative. Immunodiffusion (ID) and complement fixation (CF) are recommended as supportive tests in patients without exposure to a highly endemic area or in whom the clinical presentation is not highly suggestive of coccidioidomycosis, although either ID or CF can be used as a first-line laboratory test when locally available. Microscopy, culture, real-time polymerase chain reaction (RT-PCR), and antigen testing, as well as repeating EIA, can all be considered based on clinical suspicion and immunologic status of the patient.

Conclusion:

This diagnostic algorithm outlining a recommended approach to diagnosing pulmonary *Coccidioides* infections is intended to increase provider testing for coccidioidomycosis and, thus, reduce missed and delayed diagnoses. Feedback from Coccidioidomycosis Study Group meeting participants can help strengthen this draft algorithm before public dissemination.

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LUNG TISSUES FROM WILD SOUTHWESTERN RODENTS HELP IN UNDERSTANDING THE GEOGRAPHIC DISTRIBUTION AND DISEASE ECOLOGY OF COCCIDIOIDESAND OTHER MEMBERS OF THE ONYGENALES

Paris Hamm[1], Kyana Montoya[1,2], John Taylor[3], Schuyler Liphardt[1], Joseph Cook[1,4], Donald Natvig[1]

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Abstract

Introduction. Coccidioidomycosis (Valley Fever) is a disease of humans and animals caused by species of the dimorphic fungus *Coccidioides* and is highly endemic to arid regions of the southwestern United States. Modern genetic and ecological studies provide support for the hypothesis that small mammals are an environmental reservoir for species of *Coccidioides*. Our multifaceted approach to study the lung mycobiome of wild rodents across New Mexico, Arizona, and California documents the distribution and ecology of species of *Coccidioides*.

Methods. Ultrafrozen lung tissues were obtained for 36 species representing five families of rodents across the southwestern U.S. from the University of New Mexico Museum of Southwestern Biology and the University of California Berkeley Museum of Vertebrate Zoology. A combination of next-generation sequencing and culturing methods were used to examine the lungs for fungi.

Results. To date, we have examined the lungs of approximately 100 small mammals usingIllumina ITS2 sequencing. Sequences for species of *Coccidioides* were present in 15% of the samples, albeit often in low relative abundance. Sequence similarity analyses point to the presence of *C. posadasii* in rodent lungs from New Mexico and Arizona, reflecting results obtained from analyses of clinical isolates. *Blastomyces parvus* was present in 50% of the samples generally in low abundance, except for one lung sample with 96% relative abundance. Lung tissue from 40 small mammals have been examined using culture methods and Sanger sequencing of ITS rDNA. Plated fragments from 39 (97.5%) lung samples produced one or more fungal colonies. Cultured fungi were diverse and represent multiple Ascomycota and Basidiomycota groups. Among members of the Onygenales, we isolated cultures of *Blastomyces parvus* (9) and *Emmonsiellopsis sp*. (1).

Conclusions. Despite the fact that species of *Coccidioides* are among the few fungal pathogens to infect healthy humans, our results suggest that *Coccidoides* may be more common in hosts than expected. The lung mycobiome community in small mammals appears to often include members of the Onygenales, a conclusion where modern sequencing studies and decades-old culture studies now meet.

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MOUSE MODEL OF A HUMAN STAT4 POINT MUTATION THAT PREDISPOSES TO DISSEMINATED COCCIDIOMYCOSIS (DCM)

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Abstract

INTRODUCTION:
Previously we described a 3-generation family carrying a single missense mutation in STAT4 resulting in a GLU to GLY change at AA 626 (E626G). All individuals carrying this mutation experienced DCM. We have engineered this mutation in a genetically modified mouse.

MATERIALS AND METHODS:
C57Bl/6NJ (B6) mice were generated with the Stat4-E626G mutation and maintained as homozygotes (B6-*Stat4*$^{E626G/E626G}$). Homozygous B6-*Stat4*$^{E626G/E626G}$ were crossed to either WT B6 or DBA2 mice resulting in B6-*Stat4*$^{E626G/+}$ and B6D2 F1-*Stat4*$^{E626G/+}$ mice respectively. These mice along with WT B6 and D6D2 F1 were then inoculated intranasally with 50 spores of a slower growing *Coccidioides posadasii*, strain 1038 (Cp1038). Mice were followed for disease progression for 100 days. Cellular phenotyping of spleens, lung, and mediastinal lymph nodes and cytokine production in the lungs were determined.

RESULTS:
B6-*Stat4*$^{E626G/+}$ as well as B6D2 F1-*Stat4*$^{E626G/+}$ mice have increased susceptibility to Cp1038 infection compared to WT B6 and B6D2 F1 mice despite normal expression of Stat4 and normal development of lymphoid and myeloid compartments. WT B6D2F1 mice were able to control infection and survived the entire length of the experiment with no outward signs of disease and low lung fungal burdens upon sacrifice. WT B6 mice showed protracted disease progression and had a mean time to death of 79 days. B6-*Stat4*$^{E626G/+}$ had a rapid disease progression with a mean time to death of 40.5 days ($p<0.0001$). B6D2 F1-*Stat4*$^{E626G/+}$ mice also had progressive disease with a mean time to death of 94 days ($p=0.0294$) Fourteen days post infection B6-*Stat4*$^{E626G/+}$ mice had fewer B cells and activated T cells in the mediastinal lymph nodes. At later times cell composition of B6-*Stat4*$^{E626G/+}$ and B6 were similar in all organs. Despite similar cell numbers, cells from infected B6-*Stat4*$^{E626G/+}$ lungs produced less IFN-γ on days 28, 35 and 42 after infection. IFN-γ-stimulated cytokines (IP-10, MIG, MIP-2) were also significantly reduced compared to infected WT B6 mice. In other experiments, we crossed B6-*Stat4*$^{E626G/E626G}$ to both WT B6 and B6-*Stat4*$^{null/null}$ to create heterozygotes for E626G as well as crossing B6 to B6-*Stat4*$^{null/null}$ to create *Stat4* hemizygotes to account for gene dosage. All mice containing an E626G allele were equally more susceptible to Cp1038 infection as compared to B6-*Stat4*$^{null/+}$ and WT B6 who were also the same. Thus, the E626G mutation acts as a dominant negative.

CONCLUSION:

B6-*Stat4*$^{E626G/+}$ mice have reduced IFN-γ production and transiently reduced accumulation of immune cells in the draining lymph node following Cp1038 infection. The *Stat4*^{E626G} mutation leads to a more rapid disease progression, even in resistant B6D2F1 mice. These findings demonstrate the immunologic effects of the mutation as well as that *Stat4*^{E626G} functions as a dominant negative as a single copy of the mutation is sufficient to induce susceptibility to *Coccidioides.*

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Coccidioidomycosis and COVID-19: A Single Center Case Series

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Abstract

INTRODUCTION
To date, there has been little literature on patients with dual infection of SARS-CoV-2 (COVID-19) and *Coccidioides*. In 2020, Arizona experienced simultaneous epidemics of COVID-19 and coccidioidomycosis (cocci). In this study we aimed to describe patients from our institution with dual infection and identify risk factors for patients whose coccidioidomycosis had an unexpected manifestation or worsened after experiencing COVID-19.

METHODS
After receiving IRB approval, we conducted a retrospective review of patient electronic health records for patients with coccidioidomycosis and COVID-19 between January 1 and December 31, 2020. Cases were identified using a search for ICD-10 codes for both infections. Inclusion criteria for COVID-19 required a positive antigen or PCR test. Inclusion criteria for coccidioidomycosis required a patient to have proven or probable coccidioidomycosis, a positive serology and appropriate symptoms or positive serology in immunosuppressed host, or active follow up to monitor prior coccidioidomycosis. Outcomes of interest included progressive or relapsed cocci [increased complement fixation (CF) titer of ≥ 2 dilutions, progressive or relapsed radiographic coccidioidal abnormalities], or the unanticipated discovery of coccidioidomycosis prolonging COVID-19 symptoms.

RESULTS

From 1/1/2020 – 12/31/2020, 168 potential subjects were initially identified and 41 met inclusion criteria. Among the 41, 27 (65.9%) were male, median age 54 years (range 17-82), 31 (75.6%) non-Hispanic Caucasian, 17 (41.5%) immunosuppressed, and 29 (70.7%) had pulmonary coccidioidomycosis. Among the 41, 7 (17.1%) had simultaneously-identified (or near-simultaneous) coccidioidomycosis and COVID-19, 3 (7.3%) developed coccidioidomycosis months after COVID-19, and 31 (77.5%) had coccidioidomycosis before COVID-19. Among the 31 with prior coccidioidomycosis, 5 (16.1%) had progressive or relapsed cocci during or following the COVID-19 episode. There was no demographic (age, race, sex) or preexisting comorbid characteristic (immunosuppression, ongoing antifungal treatment for coccidioidomycosis) or the use of dexamethasone for covid-19 treatment that increased or decreased the likelihood of progressive or relapsed coccidioidomycosis during the episode of COVID-19.

CONCLUSION
Simultaneous coccidioidomycosis and COVID-19 infections were not uncommon in 2020. Further study is needed to better understand risk factors for relapsed or progressive coccidioidomycosis in the setting of COVID-19 infection or its treatment.

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EPIDEMIOLOGY OF COCCIDIOIDOMYCOSIS-ASSOCIATED HOSPITALIZATIONS AND IN-HOSPITALDEATHS, CALIFORNIA, 2000–2017

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Abstract

BACKGROUND: Coccidioidomycosis (CM) is caused by inhalation of spores of the soil-dwelling *Coccidioides* spp. fungus; infection can lead to severe respiratory or disseminated disease. In California, reported cases increased 222% since 2014 (2,316 cases) peaking in 2017 with 7,466 cases (rate 18.1/100,000 population), the highest annual reported cases on record. We reviewed California hospital Coccidioidomycosis data to describe trends, demographics, comorbidities, and risk factors for in-hospital death.

MATERIALS AND METHODS: Using 2000–2017 California administrative hospital discharge data, we identified hospitalizations with ≥1 Coccidioidomycosis-associated International Classification of Diseases, Ninth (ICD-9) or Tenth (ICD-10) diagnosis code. We calculated incidence rates per 100,000 population, assessed trends by negative binomial regression, and compared patient characteristics for potential risk factors for in-hospital death by calculating age adjusted odds ratios (aOR) using bivariate logistic regression (significance, p-value <0.05).

RESULTS: From 2000–2017, 25,372 patients were hospitalized with a coccidioidomycosis discharge code in California, and hospitalization rates increased significantly from 2.3 to 5.8/100,000 population (p<0.01). Most patients were male (69%), >40 years old (69%), white (40%) or Hispanic (38%), or residents of the higher incidence coccidioidomycosis regions in California (52%). Most (83%) were not immunocompromised; only 3% had a human immunodeficiency virus (HIV) diagnosis. A total of 1,951 (8%) patients died in-hospital with more deaths among those with disseminated coccidioidomycosis (15%), particularly meningitis (17%), than with pulmonary disease (7%). Frequency of death increased with increasing age (0-19 years [2%], 20–39 years [5%], 40–59 years [7%], 60+ years [13%]). Odds of in-hospital death was highest among patients with HIV (aOR 6.4, 95% CI 5.3–7.7) or chronic kidney disease (aOR 2.6, 95% CI 2.3–2.8).

CONCLUSION: Coccidioidomycosis-associated hospitalization rates have increased in California in the last 18 years, peaking in 2017, with 1 in 12 patients dying in-hospital. Risk factors for death include disseminated coccidioidomycosis, older age, HIV infection, and chronic kidney disease. Clinicians should be aware of these risks in caring for patients hospitalized with coccidioidomycosis.

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COCCIWATCH: PRELIMINARY RESULTS OF AIR MONITORING FOR COCCIDIOIDES SPORES IN ARIZONA AND CALIFORNIA

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Abstract

INTRODUCTION

Prevalence as well as distribution of *Coccidioides* arthroconidia (spores) in ambient air remain completely unknown. We have developed a novel approach for detection of airborne *Coccidioides* and used it to understand the seasonal and spatial distribution of *Coccidioides* in ambient air the endemic areas of Arizona and California.

METHODS

Air-monitoring collectors were deployed at 11 locations around Phoenix, AZ and at three locations in Kern County, CA, and filters were collected daily. DNA is extracted from filters and tested for the presence of *Coccidioides* DNA using previously developed qPCR assays.

RESULTS

We will present preliminary testing results from five sites in Arizona from filters collected between January 2018 and July 2019 as well from three sites in California from filters collected between August and December 2019 that demonstrate uneven spatial distribution of *Coccidioides* arthroconidia in the air.

CONCLUSIONS

Our results demonstrate that routine air monitoring for *Coccidioides* spores is possible and provides an important tool for *Coccidioides* surveillance, which can address several important epidemiological questions about environmental exposure and human infection.

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Recent Soil Analysis Calls for an Updated Realized Niche Map of *Coccidioides* spp. in the Western United States

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Abstract

Introduction

Coccidioides is a genus of understudied fungal pathogen that is the etiological agent of the infectious disease, Valley fever. Historically, these fungi are thought to be endemic to the southwestern United States; however, this evidence is generally based upon human case data and not the presence of the fungi in the environment. The objective of this study was to use 9 years of environmental presence data to evaluate the impact climate has on the realized ecological niche of *Coccidioides* species.

Methods

We used maximum entropy ecological niche modeling based on soil presence (real-time qPCR positive) data collected from 2013-2021. This data includes novel sampling locations that to our knowledge have never been examined for the presence of *Coccidioides*. We used Bioclimatic as well as soil data as predictors in our model.

Results

Results show maximum temperature of warmest month, precipitation of coldest quarter, mean temperature of driest quarter, precipitation of wettest month, and mean temperature of wettest quarter are significant bioclimatic variables in determining the niche of the fungi. Soil pH, organic carbon concentration, percent clay, are significant soil variables. In addition to the traditional hot spots in southern/central Arizona, central California our model shows a substantial probability that areas throughout the western United states have habitat that can support *Coccdioides* fungi presently or in the future.

Conclusion

The documentation of an accurate realized niche can aid in future modeling, surveillance, and give public health officials more knowledge of where hot spots are and where outbreaks can occur.

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Using precipitation to forecast coccidioidomycosis incidence in the San Joaquin Valley of California

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Abstract

INTRODUCTION
The San Joaquin Valley of California has some of the highest coccidioidomycosis incidence levels in the United States. Seasonal changes in climate conditions, especially precipitation, may help forecast the number of cases each year. We used antecedent precipitation to create an early warning system for monthly coccidioidomycosis incidence in the San Joaquin Valley.

MATERIALS AND METHODS
We used monthly, county-level cases of coccidioidomycosis from 2000-2015 to train our model and 2016-2018 cases to test our model. We accounted for the delayed response between precipitation, fungal growth, and case reporting by considering lagged responses between precipitation and changes in disease incidence. We converted cases to monthly incidence, then detrended incidence to account for changes that are less likely related to climate and more likely related to changes in disease awareness and reporting. We define the San Joaquin Valley of California as Fresno, Kern, Kings, San Luis Obispo, and Tulare Counties. We explored surface air temperature and precipitation variables from the PRISM Oregon State dataset.

RESULTS
Although we examined climate-disease relationships between both surface air temperature and precipitation, we found measures of precipitation to be the best predictors of monthly incidence. Our predictive model consists of two precipitation variables: the squared term of an 8-month moving average of precipitation that is lagged 5 months prior to incidence, and a 12-month moving average of precipitation that is lagged 12 months prior to incidence. Our model successfully captures much of the monthly variability of disease incidence; compared to observed incidence, our model had an adjusted R^2 of 0.56 for 2000-2015. Our model also captures the seasonal cycle of incidence (R^2 = 0.94). We tested our model by predicting monthly incidence for 2016-2018, to which out model performed with an R^2 of 0.65.

CONCLUSION
We created a predictive model of monthly coccidioidomycosis incidence in the San Joaquin Valley of California using lagged precipitation. This model can be used as an early warning system for public health agencies and physicians to understand the risk of an exceptional coccidioidomycosis season based on antecedent rainfall, providing warning five months in advance.

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Children are our future: Educating youth to reach adults about risks, prevention, and treatment of Valley Fever

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Abstract

Introduction: Coccidioidomycosis (also called Valley Fever) is a public health issue with 7,466 new cases reported in California in 2017. Los Angeles County (LAC) reported 1,001 cases in 2017, with the highest incidence rates occurring in the desert areas of the Antelope (AV), Santa Clarita (SCV), and San Fernando (SFV) Valleys. While children are less likely to contract the disease as compared to adults, they can be a valuable resource for educating and motivating their parents and other family members. In 2016, the LAC Department of Public Health (DPH) established a comprehensive campaign to educate children ages 8-10 about Valley Fever and to pass on this information to their families.

Methods: In 2016, LAC DPH developed a wide array of entertaining and interactive health education materials intended to both inform and engage school-aged children about the risk, prevention and treatment of Valley Fever. Outreach was conducted at two elementary schools in highly affected areas. The participating children were encouraged to pass on the information they learned to their families. Valley Fever health education materials and a follow up survey were sent home with the children, who were encouraged to share what they had learned with their families. Investigators also asked the students to complete a short survey to assess whether the information reached the children's families.

Results: Out of 369 surveys that were distributed to students, 30% (n=112) were returned. Of these, 50% (n=57) of respondents reported that they had heard of Valley Fever before. 96% (n=107) stated that the child had shared with them the information they gained regarding Valley Fever. The child shared "What Valley Fever Is" 94% (n=101) of the time, "The signs of Valley Fever" 93% (n=99) of the time, "How you prevent Valley Fever" 81% (n=87) of the time, "how Valley Fever is treated" 82% (n=88) of the time, and 77% (n=82) of respondents reported that the child shared the health education materials with them.

Conclusions: LAC DPH successfully provided Valley Fever education to 369 4th grade students in areas that are highly endemic for Valley Fever. Furthermore, the intervention demonstrated that providing interactive education to school-aged children can be a successful strategy in disseminating education about Valley Fever to parents who may not be easily reached through typical DPH communication strategies.

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Comparison of Gene Expression in the Lungs of Resistant and Susceptible Inbred Mice Infected with Coccidioides immitis

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Abstract

Introduction. Inbred mice differ in their susceptibility to inhalation coccidioidomycosis; DBA/2 are the most resistant and C57BL/6 (B6) are one of the most susceptible strains. One differences between them is both the amount of expression of Dectin 1 (the beta glucan receptor) and the length of that lectin receptor. Much remains to be learned about the different ways the two mice respond to this infection.

Methods. In this IACUUC-approved study mature female mice were anesthetized and infected intra-nasally with ~50 arthroconidia of *C. immitis* RS. After 14 days they were euthanized, and pieces of each lung from 3 randomly chosen mice from both strains were immediately frozen on dry ice for RNA extraction. The remainder of the lungs were homogenized, serially diluted with saline, and cultured quantitatively on Sabouraud dextrose agar. Lungs from two uninfected mice of the same age were similarly frozen as negative controls. Total RNA was extracted from the frozen tissues using Trizol-based extraction. mRNA was isolated using Dynabeads Oligo (dT). RNA-seq libraries were prepared using NEBNext Ultra Directional RNA Library Prep Kit and sequenced using NextSeq 500. Sequencing data was mapped to *C. immitis* RS genome using Kallisto, differentially regulated genes were identified using EdgeR.

Results. B6 mice had a hundred-fold more organisms in their lungs, as expected. Comparisons were made between gene expression in control and infected lungs, and the differential expression of genes in infected B6 and DBA/2 lungs. We considered ≥ 4-fold differences in transcript levels and false discovery rates of ≤0.01 to be significant if the gene was differentially regulated between control versus infected lungs.

The interleukin 1 family (IL-1 α and β and their receptors and receptor antagonists), and TNF and its receptor were more highly expressed in DBA/2, indicative of increased inflammation. There was evidence of increased macrophage activation with upregulation of multiple MMP genes and inducible NO synthase and arginase1, so both M1 and M2 macrophages were more abundant. IFNγ and Stat1 were more abundantly expressed implying a more robust Th1 immune response. There also was abundant expression of macrophage and T cell chemokines and their receptors including CXCL 9, 10, and 11, which are IFNγ stimulated genes.

Conclusions. Comparison of gene expression in the lungs of two mouse strains that differ in susceptibility to coccidioidomycosis revealed many differentially regulated genes that are likely very important in determining the outcome of this infection. Further experiments are needed to confirm these differences on cellular and protein levels. These could provide clues to the differences in the immune response of humans to this infection.

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A Randomized, Double-blind, Placebo-controlled Clinical Trial of Fluconazole as Early Empiric Treatment of Coccidioidomycosis Pneumonia (Valley Fever) in Adults Presenting with Community-Acquired Pneumonia in Endemic Areas (FLEET-Valley Fever)

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Abstract

Introduction: Coccidioidomycosis is a fungal infection endemic in the southwestern United States (US). Primary pulmonary coccidioidomycosis (PPC) is a leading cause of community- acquired pneumonia (CAP) in this region, although its diagnosis is often delayed, leading to lag in antifungal treatment and subsequent morbidity.

Methods: Phase IV randomized, double-blind, placebo-controlled trial in individuals aged 18 years or older with CAP who met all eligibility criteria in Coccidioides endemic regions in the US. Eligible participants with CAP were randomized to receive either fluconazole (400mg daily) or matching placebo for 42 days and were subsequently monitored for clinical resolution of their illness.

Objectives: The primary objective was to assess the clinical response of early empiric antifungal therapy with fluconazole through Day 22 in subjects with PPC who were adherent to the study intervention. Secondary objectives included: assessments of the impact of early empiric antifungal therapy with fluconazole through Day 22 and 43 in subjects with PPC regardless of adherence, comparisons of the clinical response and its individual components over time by treatment group in subjects with PPC, assessments of days lost from work or school, hospitalization, and all-cause mortality.

Discussion: This trial was halted early due to slow enrollment (72 participants in one year, 33 received fluconazole and 39 received placebo). Of those enrolled, eight (11%) met the study definition of PPC. In order to address critical questions in the field a two-step observational study was initiated with a primary objective to assess the prevalence of PPC in patients with CAP in endemic areas and key secondary objectives to determine factors that predict PPC, describe the practice of empiric antifungal treatment and to compare outcomes of patients with different therapeutic management approaches. We will review the design and current enrollment for this ongoing study.

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The air mycobiome is decoupled from the soil mycobiome in the San Joaquin Valley *Coccidioides immitis* endemic region

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Abstract

Introduction

The air and soil mycobiome in regions where *Coccidioides* is prevalent is poorly characterized. Understanding the general relationship between soil fungi and airborne fungal spores in these regions establishes a framework whereby hypotheses about *Coccidioides* dispersal can be made.

Methods

Soil samples from rodent burrows and passively deposited dust samples were collected monthly for one year at five uncultivated sites in Kern and Kings County, between Taft and Avenal, along the western edge of the San Joaquin Valley (Hwy33 samples). These were compared to agricultural soil samples and passively deposited dust samples, including previously published data, collected at the Kearney Agricultural Research Extension near Fresno (KARE samples). In both cases, the fungal community was described by sequencing the ITS2 region, followed by visualization using principal components analysis and partitioning variance among factors using PERMANOVA.

Results

The main finding of this work is that the fungal air communities are far more similar to one another than they are to the soil communities from where they were collected.

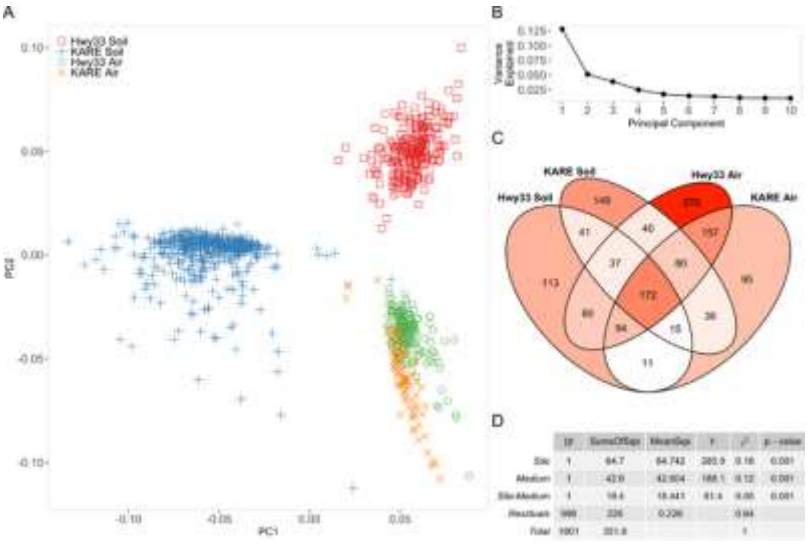

Figure 1. PCA analysis of the fungal community (A). PCA variance by component (B). Species shared across each site and sampling medium combination (C). PERMANOVA coefficient table (D).

Conclusion

First, if the air mycobiome is decoupled from the local soil mycobiome, and relatively similar across the San Joaquin Valley, it is possible that inhalation and infection by *Coccidioides* may be equally possible regardless of where you are in the region. Alternatively, it can be argued that infection is equally unlikely across the San Joaquin Valley, and that local disturbances may be required to cause infection. The numerous reports of *Coccidioides* infections associated with roadwork, root and bulb crop farm work, solar installation construction and other activity involving soil excavations indicates that local disturbance is probably key for increased similarity between local soil and air mycobiome communities, which can be extrapolated to a higher likelihood of possible *Coccidioides* infection.

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RECOMBINANT (GCP-rCpa1) VACCINE INDUCED IMMUNITY AGAINST COCCIDIOIDOMYCOSIS IN HUMANS

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Abstract

Introduction: Vaccination against coccidioidomycosis is feasible as patients can develop life-long immunity to this mycosis. We utilized a recombinant antigen (rCpa1) enveloped into glucan-chitin particles (GCP) as an adjuvant-delivery system in order to elicit protective immunity against coccidioidomycosis. Effective vaccine immunity in mice is largely dependent on fungal detection by C-type lectin receptors that prime $CD4^+$ T cells, particularly Th17 cells. Our objective is to characterize human immune responses of Antigen Presenting Cells and T cells to this newly created GCP-rCpa1 vaccine.

Materials and Methods: Human monocyte-derived macrophages (hMDM) and enriched $CD4^+$ T cells were prepared from PBMCs of healthy volunteers (South Texas Blood and Tissue Center). The global gene expression profiles of hMDMs that were exposed to GCP-rCpa1 were quantified by RNA-seq and Ingenuity Pathway Analysis (IPA). Mature THP-1 Dual NF-kB reporter cells (InVivogen) were co-cultured with GCP-rCpa1 for 24 hr and NF-kB activation was quantified via SEAP reporter system. The primary hMDMs were co-cultured with autologous $CD4^+$ T cells in the presence of GCP-rCpa1 for 24 hr, washed and then cultured in serum-free TexMacs™ medium to expand T cells for 6 days. Human T-cell responses were measured by flow cytometry and cytokine assays after they were restimulated with rCpa1 or one of the component peptides at a concentration of 100 nM.

Results: RNA-seq analysis by GCP-rCpa1 stimulated hMDM revealed upregulation of both Th1 and Th17-associated genes (*TNF-a, IL-1b, IL-6*, and *IL-17*). Additionally, quantitative real-time PCR analysis confirmed upregulation of these genes. IPA analysis predicted that hMDMs could recognize this vaccine via TLR1/2, TLR2/6 and Dectin-1 receptors. THP-1 Dual reporter cells showed that in the absence of MyD88 and CARD9 silencing NF-KB production is dampened. Antigen presenting molecules (MHC II and CD86) of hMDMs were significantly increased in 12 subjects after exposure to the vaccine compared to PBS alone. Next, we studied immune response of human $CD4^+$ T cells that were co-cultured with the vaccine-primed hMDMs. Surprisingly, human T cells that were re-stimulated with rCpa1 demonstrated an ex-Th17 (non-classical Th1) phenotype that they were $CCR5^-CCR6^+IFN\text{-}\gamma^+IL\text{-}17^-$.

Conclusion: These results reveal that the GCP-rCpa1 vaccine elicits a mixed Th1/Th17 response both in mice and humans. Recognition of adjuvant may be attributed to TLR and CLR detection in humans. While vaccine-induced Th17 cells in the murine lungs continue to express IL-17, the human Th17 cells switch to become non-classical Th1 cells that produce IFN-γ *in vitro*. The differences between murine and human T-cell response to this newly created vaccine will be focused on the characterization of their immune function and the identification of T-cell epitopes in the rCpa1 antigens

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Coccidioidomycosis in horses: a case series and review of the literature

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Abstract

INTRODUCTION: Horses have fairly low susceptibility to coccidioidomycosis; however, some breeds of horses, as well as immunocompromised horses, are more susceptible, and medical treatments can last months leading to high medical bills, or when disease is severe, to death. The scientific literature on coccidioidomycosis in horses is limited, so herein we present a retrospective study of 28 coccidioidomycosis cases in horses, including the organ distribution and description of the lesions.

METHODS: The records of the California Animal Health and Food Safety (CAHFS) laboratory system were searched for equine autopsy cases with a diagnosis of coccidioidomycosis that were submitted between January 1st 1990 to January 31th 2020. The selection criteria were: i) horses submitted for autopsy for which ii) a diagnosis of coccidioidomycosis was established, regardless if this was the cause of death or not. In all cases, samples from liver, lung, kidney, spleen, skeletal muscle, skin, adrenal gland, small intestine, colon and/or brain had been collected, fixed in 10% buffered formalin for periods variable between 24 hs and a week, and processed routinely for the production of HE sections. The prevelance of coccidioidomycosis was determined in the study group. All autopsy reports of horses diagnosed with coccidioidomycosis were evaluated for significance and organ distribution of disease. Coccidioidomycosis was determined to be either the cause of death or incidental if another cause of death was identified. As coccidioidomycosis commonly affected the lung/pleura or horses, all horses diagnosed with pneumonia during the study period were also evaluated to determine the prevelence of coccidioidomycosis pneumonia versus other types of pneumonia. The prevelance of coccidioidomycosis abortion was also determined.

RESULTS:

The prevelance of coccidioidomycosis in the study group horses was 0.15% (28/19,054)

Of the total number of coccidioidomycosis cases (n=28), the condition was an incidental finding in 14 cases and the cause of death in the other 14 cases. 1.2% of pneumonias during this period were caused by *Coccidioides* spp. and 7 of those coccidioidomycosis pneumonias (0.5%) caused the horses death. Disseminated coccidioidomycosis (involving two or more organs) was identified in 4 of the 28 (14%) horse with coccidioidomycosis. Aside from the lung or disseminated disease, the remaining cases only involved one organ, which included spleen (n=1), nasal mucosa (n=1), placenta (n=2), and thoracic cavity lymph nodes (n=3). Three abortions out of 1,896 total equine abortions (0.15%) were caused by coccidioidomycosis.

CONCLUSION: Coccidioidomycosis is rare in horses and appears to be an incidental finding in about half of the reported cases. It most commonly affected the pulmonary system of horses, followed by disseminated disease, which was the other main form a disease leading to death. Our results are consistent with findings in other species, indicating that the respiratory route is the main portal of entry for *Coccidioides* spp. in horses.

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Genomic Epidemiology of *Coccidioides* Links Non-Endemic Coccidioidomycosis to Travel

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Abstract

INTRODUCTION: Coccidioidomycosis is an illness endemic to specific warm, arid regions in the Americas. An increasing number of sporadic cases outside these regions are either travel-associated or contracted in as-yet unknown locales of the etiologic soil fungus, *Coccidioides*. A previous analysis of cases in non-endemic regions identified epidemiological indicators that the infections could have been acquired during travel for the majority of the cases. However, this finding was based purely on reported patient travel history.

METHODS: To further investigate the likelihood of travel related infections in these cases we sequenced the genomes of 19 *Coccidioides* isolates from patients in non-endemic regions with travel to confirmed or suspected endemic locales, to ascertain phylogeography to assign likely exposure site.

RESULTS: Our analyses indicate that although patients may have traveled to more than one potential endemic site, their respective *Coccidioides* isolates are phylogenetically linked to *Coccidioides* subpopulations naturally occurring in one of their reported travel locales.

CONCLUSION: Although additional *Coccididioides*-endemic regions have recently been discovered (e.g., southcentral WA), most non-endemic coccidioidomycosis is likely acquired during travel to endemic regions, and can be confirmed through genomic epidemiological investigations.

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Metabolic reprogramming and Immune response reflecting disease spectrum in patients with Coccidioides infection and with SARS-CoV-2 co-infection

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Abstract

Introduction: *Coccidioides* infection leads to acute or chronic pulmonary disease, Valley Fever (VF) with a wide spectrum of disease severity. However, mechanisms contributing to coccidioidomycosis disease severity and therapeutic failures are not fully elucidated and correlates of disseminated or unresolved infection are not well established. SARS-CoV-2 causes acute or chronic pulmonary infections and shares common features of mucosal transmission, pulmonary disease and high incidence in similar geographic locations with *Coccidioides*. The overall objective of our study was to investigate the immune and metabolic correlates of *Coccidioides* infection spectrumand to identify mechanisms of disease patho genesis. We also investigated the impact of SARS-CoV-2 co-infection asa confounding factor for impairing host immune and metabolic response to these pulmonary pathogens.

Material and methods: Serological assays were utilized to determine the host immune response to *Coccidioides* (detection of IgG and IgM by immunodiffusion and complement fixation tests) and to SARS- CoV2 (detection ofantibodies against nucleocapsid and spike protein using an enzyme-linked immunosorbent assay). Serum samples were obtained from 18 VF-negative healthy controls, 25 VF individuals (VF+/COVID-), 23 co-infected individuals (VF+/COVID+) and 104 SARS-CoV2 infected individuals (VF-/COVID+). Cerebrospinal fluid samples from 25 VF patients and 31 patients with VF associated meningitis were collected. Untargeted metabolomic analysis was performed using Ultra- Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS)(Metabolon). This study was approved by the Institutional Review Board.

Results: Substantial host metabolic reprogramming was induced in *Coccidioides* infection as detected by marked changes of metabolic profiles in serum and CSF samples. Changes in serum samples demonstrated increasedlipolysis, inflammation, oxidative stress, mitochondrial dysfunction and coagulopathy during VF. These changeswere further exacerbated in the CSF of VF meningitis (VFM) patie nts. CSF metabolomics of VFM patients revealed additional changes related to central nervous system damage and inflammation and were evidenced by decreasedglucose metabolism and ac tivation of kynurenine pathway. We have identified a metabolic panel that can be used as a biosignature for diagnosis of VF and disseminated VFM. Metabolomic profiling showed that patients coinfected with *Coccidioides* and SARS-CoV2 had impaired functional networks that were similar to those in disseminated VF meningitis patients. Serological data showed the impact of *Coccidioides* and SARS-CoV2 coinfections on the hosthumoral immune response.

Conclusion: We have identified a candidate metabolic signature for *Coccidioides* infection that can be us ed for monitoring disease spectrum. We also found that coinfection with SARS-CoV-2

accentuated metabolomic changescontributing to cellular and mitochondrial dysfunction. Collectively, o ur studies fill gaps in our understanding of thepathogenesis of Valley Fever disease spectrum and the infl uence of SARS- CoV-2 co-infection on functional outcomes. Our findings will help in identifying potential correlates of disease outcomes and pathways as therapeutictargets.

Poster Presentation Abstracts

3

Beta-D-Glucan Testing for Diagnosis of CNS Coccidioidomycosis

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Abstract

Introduction: Detection of the fungal cell wall component (1-3) β-D-glucan (BDG) in body fluids is often used as a marker for the presence of fungal infection. This method has been successfully utilized to aid in identifying many types of fungal infections, including coccidioidomycosis (CM). CM is caused by the dimorphic fungal pathogen *Coccidioides immitis* and *posadasii* which are endemic to regions within the Southwestern U.S. Disease severity ranges from asymptomatic to potentially life-threatening disseminated disease, such as meningitis. Detection of BDG has sometimes been indicated to identify fungal meningitis. Detection of *Coccidioides* galactomannan (GM) in cerebrospinal fluid (CSF) has already been established in diagnosing meningeal coccidioidomycosis.

Methods: A total of 25 CSF specimens from individuals with confirmed meningeal coccidioidomycosis and 35 otherwise healthy individual specimens were used as negative controls. Samples were frozen at MiraVista Diagnostics and tested for BDG in the FDA cleared Fungitell™ assay [1] according to established procedures.

Results: The sensitivity of the MiraVista *Coccidioides* antigen enzyme immunoassay (EIA) was 88% compared to 68% for the BDG assay (p=0.0625) and the specificity was 100% and 74.3% respectively (p=0.003). Correlation of BDG and *Coccidioides* galactomannan antigen concentration showed poor correlation by linear correlation, r^2=0.3096.

Conclusion: The Fungitell™ BDG assay is neither sensitive nor specific for diagnosing *Coccidioides* meningitis and is inferior to the MiraVista *Coccidioides* GM assay.

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CAVITARY COCCIDIOMYCOSIS IN PATIENTS WITH DIABETES MELLITUS

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Abstract

Introduction:

The clinical manifestations of coccidioidomycosis (CM) may vary by innocolum, the pathogenicity of particular strains and immune status of the host. Diabetes, particularly uncontrolled diabetes has demonstrable adverse effect on many aspects of the immune response. Infections in diabetics may be exacerbated by any number of immune alterations. Individuals with diabetes mellitus (DM) are more likely to experience severe CM. Cavities occur as a complication of primary lung disease and present in 2-3% of population.

Cavitation in these patients represents chronic disease and clinicians use size, location, wall thickness, and number to characterize them. The purpose of this paper is to explore the frequency, severity, location, wall thickness in patients with cavitary CM in diabetics.

Methods:
Approval was obtained from the Institutional Review Board, Kern Medical. Patients with diabetes mellitus and coccidioidomycosis in the last 10 years were identified with ICD-9 codes. A retrospective chart review was conducted with proven pulmonary CM and DM. History, physical exam, laboratory data and imaging were extracted. The size, location, wall thickness and number were recorded. Records of 114 patients with diabetes mellitus and pulmonary CM were reviewed. Patients with diabetes were divided in 2 groups. Those with an HbA1c of 6.5-7.0 were considered to be controlled diabetics. Those with HbA1c > 7.0 were considered to be uncontrolled diabetics.

Results:
Of the 31 individuals with HbA1c between 6.5 – 7.0 one (3.2%) was found to have a cavitary lesion. Of the 83 patients with HbA1c > 7.0 fifty were found to have a cavitary lesion (60.2%).

14 of 51 patients with cavitary disease (27.5) had multiple cavitations. 38/66 (58%) lesions were found in the upper lobes, 25/66 (38%) in the lower lobes, and 3/66 (4%) were right middle lobe lesions. Cavities were exactly evenly divided in the right and the left lung. We defined size of the lesion as the single greatest dimension. The size of cavities ranged from 7 mm to 60 mm, with a mean of 26.5 mm. The median was 25 mm.

Conclusions:

In this study cavitary lesions are demonstrated to be remarkably increased in individuals with poorly controlled diabetes. The advantages of euglycemia in diabetes are well known and is conceivable that good diabetic control can have a positive effect on the outcome of coccidioidal outcome.

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Impact of COVID-19 Pandemic on Diagnosis of Acute Primary Pulmonary Coccidioidomycosis

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Abstract

INTRODUCTION:
In 2020, the COVID-19 pandemic overwhelmed healthcare systems across the world. As hospitals and clinics addressed surges in COVID cases, it is reasonable to ask whether patients with other medical conditions received time-appropriate care. Subsequent published data did indeed indicate delays and reductions in such care. In 2020, the state of Arizona experienced not only COVID-19 surges, but also high numbers of coccidioidomycosis cases. Both illnesses have similar clinical presentations. The impact of COVID-19 on diagnosis and management of pulmonary coccidioidomycosis has not been evaluated. We hypothesized that the pandemic would lead to delayed recognition and diagnosis of coccidioidomycosis. The aim of our study was to assess whether the diagnosis of acute primary pulmonary coccidioidomycosis (PPC) in 2020 was delayed when compared to 2019.

METHODS:
We conducted a retrospective chart review of patients who were diagnosed with acute PPC from March 1st through December 1st in years 2019 and 2020 at Mayo Clinic Arizona. We searched for potential subjects with the ICD-10 codes B38.0 and B38.2. We included adult subjects with acute PPC. The chart review process is still ongoing at the time of abstract submission. From each chart we abstracted patient demographics, time from symptom onset to diagnosis, number of COVID-19 tests between onset of symptoms and diagnosis (2020 only), number of in-person and video visits.

RESULTS:

From 3/1/2019 – 12/1/2019, we identified 483 potential subjects, 83 of whom met inclusion criteria; from 3/1/2020-12/1/2020, we identified 479 potential subjects, 29 of whom have thus far met inclusion criteria. There were no differences between the two groups with respect to patient demographics, tobacco use, and co-morbidities. The 2020 group had a trend to lower median number of days to diagnosis compared to the 2019 cohort (11 vs. 17 days, p=0.0518). The 2020 cohort also had a lower median number of in-person visits compared to 2019 (2 vs. 1, p=0.0378). There was no difference between the two cohorts in total number of visits to a medical provider. In 2020 cohort, the average number of COVID tests prior to PPC diagnosis was 2 (SD = 1.1).

CONCLUSION:

The 2020 COVID-19 pandemic was associated with a shorter time to diagnosis of acute PPC from the onset of symptoms compared to 2019.

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REACTIVATED COCCIDIOIDOMYCOSIS FOLLOWING GLUCOCORTICOID THERAPY: 2 PATIENTS

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Abstract

Introduction:

In coccidioidomycosis (CM) cell-mediated immunity is required for the control of this infection. Patients undergoing immunosuppressive therapy, have a high risk of severe, primary pulmonary infection, disseminated or relapse infection. Herein is a report of two cases of coccidioidomycosis reactivated during the course of care of severe COVID-19 and chronic kidney disease status post glucocorticoid therapy.

Methods:

Approval was obtained from the Institutional Review Board, Kern Medical. Literature search was conducted on PubMed and google scholar. The following search terms were applied: Reactivation of Coccidioidomycosis, Immunosuppressive therapy and Reactivation of Coccidioidomycosis post glucocorticoid treatment. The patients are current cases seen at Kern Medical during the ongoing COVID-19 pandemic.

Results:

In January 2021 two patients with a history of glucocorticoid treatment for SARS-CoV-2 and chronic kidney disease stage 3b were admitted. Both patients were found to have miliary coccidioidal infections.

49-year-old Latinx man presented to our institution after 14 days of illness. He had bilateral infiltrates on chest x-ray and was diagnosed with severe COVID-19 in December 2020. He received empiric antibiotics and did not receive remdesevir because of symptom onset and time to admission. He did receive dexamethasone 6mg per day. By hospital day 6 he was discharged in stable condition on home oxygen and continued dexamethasone. Exacerbations of his symptoms eventuated on re-presentation to the emergency department. He was evaluated and discharged home on continuing therapy. The following day he returned and was found to be hypoxic. Radiographic evaluation revealed substantial changes including cavitation in the right lower lobe and miliary pattern, not previously noted. The miliary pattern on chest x-ray prompted a cocci serology as part of the evaluation. Subsequently he required intubation for two days. His therapy was changed to isoniazid, rifampin, pyrazinamide, ethambutol and voriconazole. When the cocci serology was found to be positive this was changed to amphotericin B liposomal 5mg/kg. By hospital day 10 he was discharged. He is continuing on liposomal Amphotericin in the outpatient infusion center.

A 52-year-old Latinx man presented with diabetes and focal segmental glomerulosclerosis, CKD 3b. He was treated with prednisone 60 mg ten days before admission. He presented to our institution with somnolence, nausea and vomiting. He had no previously known history of CM. On imaging, chest x-ray revealed diffuse micronodular densities throughout both lungs and CT abdomen revealed left lower lobe alveolar density. Laboratory evaluation was positive for CM serology with reactive ID IgM, IgG and complement fixation (CF) of 1:64. Lumbar puncture revealed WBC 665, glucose 2, protein 242, opening pressure 170 mmH2O and CF of 1:8. He was treated with high dose amphotericin B liposomal, fluconazole, and dexamethasone taper over. He deteriorated and died on day 41.

Conclusion:

Glucocorticoids are a double-edge sword. In high dose they have broad spectrum of immune suppression. This may advantage patients who are having ana excessive immune response. This intervention demonstrably benefits COVID-19 patients with severe disease. This may also be true in patients with severe pulmonary CM.

These cases demonstrate that glucocorticoids unopposed by antifungal therapy may eventuate in severe reactivation of CM. The lesson learned is that it may well be optimal to screen all patients to be placed on substantial immunosuppression for CM prospectively.

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A CASE REPORT OF COCCIDIOIDOMYCOSIS IN THE RENAL PARENCHYMA OF UNUSUAL SEVERITY

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Abstract

Introduction:

Coccidioidomycosis (CM) has been described in virtually every part of the body, including the genitourinary (GU) system. Disseminated CM to the GU tract is well-known to occur but is rarely documented. It is believed this is the first case of a renal parenchymal abscess.

Methods:

Approval was obtained from the Institutional Review Board of Kern Medical. A retrospective review of the patient's record was performed. A literature search was conducted on PubMed, Google Scholar, Centers for Disease Control and Prevention (CDC), Infectious Diseases Society of America (IDSA)'s Clinical Infectious Diseases Journal database, and American Urologic Association's, The Journal of Urology database. The following search terms were applied: Coccidioidomycosis, Genitourinary Coccidioidomycosis, Renal Coccidioidomycosis, Renal Parenchymal Coccidioidomycosis, Disseminated GU Coccidioidomycosis.

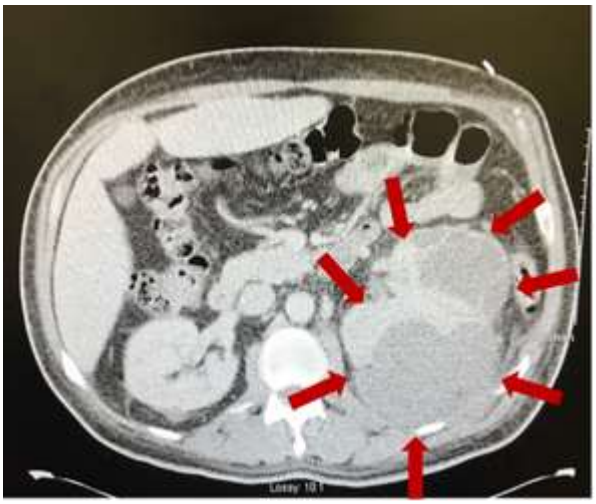
Results:

A 52-year-old Latinx man who is an oil field worker in the Southern San Joaquin Valley of California with uncontrolled diabetes mellitus. He was diagnosed with cavitary CM at another institution. Four years after his initial diagnosis, he was hospitalized at another institution for coccidioidal empyema. He underwent video-assisted thoracoscopic surgery (VATS). He presented to our institution one month later with pain fatigue and unintentional weight loss of 20 pounds. Examination was positive for left costovertebral angle (CVA) tenderness.

Laboratory examination revealed mild eosinophilia (absolute eosinophil count 383cells/microliter). Chemistry revealed a glucose of 166 mg/dL and an HbA1c 11%. Initial urinalysis revealed minimal leukocyturia. Inflammatory markers ESR 25mm/hr and CRP 3.5mg/L (≤0.3 mg/L) were elevated. Coccidioidal serologic tests revealed elevated complement fixing antibodies (≥ 1:512).

Computed Tomography (CT) demonstrated a 15x11x16cm left renal mass with cystic and solid components extending into the perinephric space. (See **Figure 1**) Fluoroscopic-guided drainage of 800cc of purulent fluid was performed. Aspirate grew *coccidioides immitis*. Antifungal therapy was initiated with 800mg of oral fluconazole once daily.

He remained adherent to antifungal therapy (oral fluconazole 800mg daily) for 36 months. His coccidioidal CF serum titers improved from 1: (≥ 512 to <1:2. Fluconazole levels were monitored regularly. His coccidioidal renal abscess was resolved clinically and radiologically. He is currently in post-therapy follow-up for relapse.



Conclusions:

The actual incidence of renal CM is currently unknown. It may be an asymptomatic manifestation or with vague upper and/or lower GU symptoms. It is probable that the most common manifestation of CM in the kidney is microscopic as granulomatous inflammation. It is our hypothesis that abscess is a very uncommon manifestation. The disease may not be revealed by blood or urine analysis. Anatomical seeding and subsequent local destruction impact disease presentation clinically and radiographically. The treatment of this case was based on treatment of other disseminated foci for which there is more experience.

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TESTING FOR COCCIDIOIDOMYCOSIS AND SARS-COV-2 BY INFECTIOUS DISEASE SPECIALISTS, UNITED STATES

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Abstract

INTRODUCTION: The clinical similarities between coccidioidomycosis and other respiratory illnesses, including COVID-19, can present diagnostic challenges; co-infection with fungi and SARS-CoV-2 is also a growing concern. Understanding how the COVID-19 pandemic has influenced infectious disease (ID) specialists' testing practices for coccidioidomycosis is essential for understanding continued challenges related to potential delayed and missed coccidioidomycosis diagnoses.

METHODS: The Emerging Infections Network (EIN) is a provider-based surveillance network supported by the Centers for Disease Control and Prevention and the Infectious Diseases Society of America. EIN emailed a 7-question poll to its >2,700 member listserv (https://ein.idsociety.org) three times during January–February 2021; 196 responded.

RESULTS: Among 191 practicing ID physicians, most (n=172, 90%) had consulted on or treated COVID-19 patients. California had the highest number of respondents (n=37, 19%), and 14 (7.3%) were from other coccidioidomycosis-endemic areas (Arizona, Nevada, New Mexico, Texas, and Utah). Before the COVID-19 pandemic, 84% of respondents in endemic areas and 18% in non-endemic areas sometimes or frequently tested for coccidioidomycosis among patients with signs and symptoms of community-acquired pneumonia; 82% of those in endemic areas and 15% in non-endemic areas said they currently do so. Highly influential factors in deciding to test possible COVID-19 patients for fungal infections included: presence of underlying condition(s) that predispose to fungal infections (54%), exposure to known endemic areas (54%), exposure to a specific environmental source (54%); presence of abnormal imaging results (24%), lack of clinical improvement (19%), and having two or more negative SARS-CoV-2 tests (14%) were less commonly noted as highly influential.

CONCLUSIONS: In endemic areas, ID physicians reported high rates of coccidioidomycosis testing both before and during the COVID-19 pandemic, relying primarily on epidemiologic exposures and host status to guide testing decisions. Although ID physicians may be more likely to see patients with coccidioidomycosis after failed treatment for other suspected infections and are thus probably more inclined to test for fungal infections than other healthcare providers in general, these results indicate that many ID physicians have continued to consider fungal infections as a cause of respiratory illness during the COVID-19 pandemic.

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Differential Thermotolerance Adaptation between Species of Coccidioides

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Abstract

Introduction: Coccidioidomycosis, or Valley fever, is caused by two species of dimorphic fungi. Based on molecular phylogenetic evidence, the genus Coccidioides contains two reciprocally monophyletic species: C. immitis and C. posadasii. However, phenotypic variation between species has not been deeply investigated. We therefore explored differences in growth rate under various conditions.

Methods: A collection of 39 C. posadasii and 46 C. immitis isolates, representing the full geographical range of the two species, was screened for mycelial growth rate at 37 °C and 28 °C on solid media. The radial growth rate was measured for 16 days on yeast extract agar.

Results: A linear mixed effect model was used to compare the growth rate of C. posadasii and C. immitis at 37 °C and 28 °C, respectively. C. posadasii grew significantly faster at 37 °C, when compared to C. immitis; whereas both species had similar growth rates at 28 °C.

Conclusion: These results indicate thermotolerance differs between these two species. As the ecological niche has not been well-described for Coccidioides spp., and disease variability between species has not been shown, the evolutionary pressure underlying the adaptation is unclear. However, this research reveals the first significant phenotypic difference between the two species that directly applies to ecological research.

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COCCIDIOIDAL PERITONITIS: A REVIEW OF 13 CASES

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Abstract

Introduction:

Though rare, coccidioidal peritonitis has been reported multiple times. Previous reports included single cases with or without reviews of literature. 31 cases have been published to date. This report describes thirteen new cases previously unreported from California.

Methods:

This study was approved by the Kern Medical (KM) Institutional Review Board. ICD 9 and ICD 10 codes were used to query Valley Fever Institute and KM's electronic health record for a period of ten years. Literature search was conducted on PubMed and google scholar using the following search terms: peritonitis, coccidioidal peritonitis, peritoneal coccidioidomycosis and omental caking. Recent cases were also submitted by our KM colleagues in Gynecology and General Surgery and infectious disease colleagues at other institutions.

Results:

Two cases were found incidentally at surgery that had no pre-surgical symptoms or imaging that suggested coccidioidal peritonitis. (Table 2.0) Eleven patients presented with non-specific abdominal complaints. They were found to have abnormal imaging or ascites that led to a diagnostic procedure which eventuated diagnosis. Two of them were diagnosed serologically without intervention. Nine underwent diagnostic procedure. The procedures consisted of paracentesis or laparoscopic evaluation. (Table 1.0, 2.0)

	Gender	Age	Ethnicity	Medical History	Presenting Symptoms	Presenting Signs	SAAG Score	Incidental Diagnosis
KM1	F	47	African American	Primary CM	Headache, loss of appetite, 100lb weight loss	None	0.2	No
KM2	M	24	Latinix	Disseminated CM, HCV	Abdominal pain	Abdominal distention	N/A	No
KM3	M	66	Latinix	HTN, ESRD w/ Hemodialysis, DM1	Headache, abdominal pain	None	N/A	No
KM4	F	57	White	Primary CM	RUQ Pain, nausea, vomiting, 15lb weight loss	None	N/A	Yes
KM5	M	52	African American	Primary CM	Abdominal pain, 15lb weight loss	Abdominal distention	0.2	No
KM6	M	33	Latinix	Primary CM	Abdominal pain	Increased abdominal girth	N/A	No
KM7	M	21	Latinix	None	Abdominal pain, nausea and vomiting	Abdominal distention	N/A	Yes
KM8	F	32	African American	Primary CM	Pelvic/flank pain	None	N/A	No
C9	M	23	Latinix	None	Nausea, vomiting, weight gain	None	N/A	No
V10	M	47	African American	HTN, Cervical Spine Stenosis	Abdominal pain, ascites, cutaneous lesions	None	0.8	No
V11	F	37	White	COPD	Abdominal pain, cutaneous lesions	Abdominal distention	N/A	No
S12	M	65	White	Eczema, Bronchitis	Fatigue, dry cough, weight loss, arthralgias, petechial rash on extremities, fever, night sweats	Abdominal distention	N/A	No
P13	M	44	Asian	Irritable bowel syndrome	Myalgia, nausea, fatigue, weight gain, poor appetite, shoulder pain	None	N/A	No

	Diagnostic Imaging & Findings	Procedures & Findings	ID IgM	IgG	CF	Antifungal Therapy	Outcome
KM1	CT Chest: upper abdominal ascites	Paracentesis	Reactive	Reactive	1:512	Initial Ambisome transitioned to voriconazole	Long-term follow-up
KM2	CT Abdomen: Ascites and pelvic abscess	Paracentesis abdominal fluid – Gram stain grew *coccidioides immitis*	Reactive	Reactive	1:512	Initial Ambisome transitioned to isavuconazonium	Long-term follow-up
KM3	CT Abdomen: peritoneal thickening of colon wall, bladder and retroperitoneum	None	Non-Reactive	Reactive	1:128	Fluconazole	Lost to follow-up
KM4	None	Laparoscopic cholecystectomy: visualized peritoneal studding Soft tissue biopsy positive for CM	Reactive	Reactive	1:2	Initial Ambisome transitioned to posaconazole	Long-term follow-up
KM5	US and CT Abdomen: ascites	Paracentesis	Reactive	Reactive	1:128	Fluconazole	Deceased secondary to CVA
KM6	CT Abdomen: ascites	None	Reactive	Reactive	1:512	Fluconazole	Lost to follow-up
KM7	CT Abdomen with contrast: multiple fluid levels	Soft tissue biopsy: granuloma consistent with CM, no endospores seen	Reactive	Reactive	1:64	Amphotericin B	Long-term follow-up
KM8	Pelvic US: solid ovarian mass	Diagnostic laparoscopy: granuloma Soft tissue biopsy: fungus resembling *coccidioides* species	Reactive	Reactive	1:256	Fluconazole	Transferred to a different geographic location
C9	US and MRI Abdomen: ascites	Paracentesis	Reactive	Reactive	>1:8	Fluconazole for 6 months, discontinued in March 2019	Long-term follow-up
V10	CT Abdomen and Pelvis: ascites and omental studding	CT guided needle biopsy of omentum: non-malignant and GMS stain positive	Reactive	Reactive	1:128	N/A	Long-term follow-up
V11	CT Abdomen with contrast: ascites with omental caking	Paracentesis	Reactive	Reactive	1:256	Fluconazole	Long-term follow-up
S12	CT Abdomen: miliary studding	Abdominal laparoscopy: ascites and studding visualized Biopsy: fungal culture grew *coccidioides immitis*	Reactive	Reactive	1:128	Initial Ambisome transitioned to fluconazole	Long-term follow-up
P13	None	Exploratory laparoscopy: miliary studding Fungal culture: coccidioides Peritoneal biopsy: *coccidioides*	Reactive	Reactive	1:512	Isavuconazonium	Long-term follow-up

All patients were subsequently treated with anti-fungal therapy. Four patients received a brief course of amphotericin B liposomal and all patients were treated with azoles with an anticipated duration of three or more years.

Conclusions:

These case series demonstrates that CM peritonitis may be asymptomatic or present with subtle abdominal symptoms. The differential diagnosis includes tuberculosis, abdominal malignancies including ascites, mesenteric caking or mass lesions. Clearly the signs and symptoms of coccidioidal peritonitis can be extremely subtle or more obvious but may require evaluation including imaging, serologic evaluation, biopsy with histopathology and/or culture for definitive diagnosis.

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EFFECTS OF TNF-ALPHA INHIBITION ON A MOUSE MODEL OF CONTROLLED COCCIDIOIDES INFECTION

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Abstract

INTRODUCTION:
Among the biological disease-modifying antirheumatic drugs utilized for treatment of a variety of immune-mediated inflammatory diseases, the first and most commonly used are TNF-α inhibitors (TNFi) : infliximab (Remicade), adalimumab (Humira), golimumab (Simponi), and etanercept (Enbrel). All TNFi have been reported to be associated with severe *Coccidioides* infections though not all patients treated with these drugs who become infected with *Coccidioides* experience severe disease. The underlying biological mechanisms that could explain the diversity remain unknown, but could include immunogenetic diversity, previous exposure to *Coccidioides* and comorbidities. Development of a mouse model to dissect the key components in these mechanisms is an opportunity for rapid discovery.

MATERIALS AND METHODS:
C57BL/6NJ X DBA/2J (B6D2 F1) mice, which experience long term control of disease when given *C. posadasii* strain 1038, were inoculated intranasally with 50 spores. To test the effect of TNFα suppression on coccidioidal infection, anti-TNF-α monoclonal antibodies or an isotype antibody control were administered intraperitoneally (ip) twice weekly starting 2 days prior to infection and continuing until euthanasia. As a positive control for immunosuppression, mice were administered dexamethasone in drinking water. Mice were monitored for disease progression and euthanized when moribund if prior to end of study. Survival time and organ fungal burdens were assessed.

RESULTS:
B6D2 F1 mice treated with control antibody all survived the 68-day study period showing no outward signs of disease; they had a mean total lung fungal burden of 2.02×10^4 cfu and spleen fungal burdens ranging from 0-730 cfu. Mice treated with dexamethasone survived a median of 34 days [range 18-51] (p=0.0002 vs. isotype control Ab) and mice treated with anti-TNF-α mAb survived a median of 39 days [range 22-51] (p= 0.0015). Lung fungal burdens were high and not significantly different (anti-TNFα – mean 9.29×10^6, dexamethasone – mean 4.23×10^6; p=0.095), and dissemination was universal in both of these groups.

CONCLUSION:
Resistant B6D2 F1 mice infected with Cp1038 can serve as a model to examine the effect of TNF-α inhibition on *Coccidioides* disease progression. When B6D2 F1 mice are suppressed with anti-TNF-α antibody prior to infection with Cp1038 they exhibit disease progression to moribundity, high lung fungal burdens, and dissemination similar to mice receiving broad immunosuppression with dexamethasone. The fact the targeted TNF-α inhibition is similar to broad immunosuppression highlights the critical role of TNF-α in host defense against *Coccidioides*. Additionally, the model of B6D2

F1 mice with Cp1038 can serve as a platform to examine different timings of TNF-α inhibition. These mice receiving full time TNF-α intervention model naïve patients moving to an endemic area. Future experiments could model patients who have previously controlled infection before starting anti-TNF-α therapy. This model will also allow us to start examining the cellular sources of TNF-α that are critical for protective immune responses.

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Monocyte and Dendritic Cell Responses to *Coccidioides*

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Abstract

INTRODUCTION: Effective *Coccidioides* clearance requires monocyte migration into the site of infection and subsequent differentiation as well as dendritic cell activation to stimulate adaptive immunity. Macrophages mediate fungal clearance via phagocytosis and effector cytokine secretion while dendritic cells (DCs) bring antigen to naïve T cells to stimulate productive adaptive immune responses. In various antimicrobial settings, macrophages and dendritic cells polarize into specialized subsets. However, little is known about the dynamics and interactions between these immune cells and *Coccidioides* at the start of infection. Flow cytometry is a powerful tool for elucidating protein expression and combined with traditional function assays, will be used to characterize *Coccidioides* induced immune cell activation.

MATERIALS AND METHODS: *Coccidioides posadasii* NR-166, an avirulent lab strain, was cultured and processed to obtain arthroconidia suspension for mouse infections and in vitro experiments. Human monocyte U937, mouse macrophage RAW 264.7, rat alveolar NR8383, and C57BL/6 bone marrow derived monocytes (BMDM) were used in phagocytosis and differentiation assays. Cells were stimulated and infected, then stained for differentiation markers. Flow cytometry, imaging and microscopy were used to assess cellular function and activation state. C57BL/6 mice were intranasally infected with 10^5 arthroconidia and their tissues harvested for immune cell analysis via flow cytometry on day 1 and 7 post-infection.

RESULTS: Flow cytometry allowed phagocytosis analysis on several cell lines in high throughput. Monocytes poorly phagocytose *Coccidioides* compared to macrophages, and differences in phagocytosis were observed between cell lines. In response to *Coccidioides* in vitro, monocytes differentiate into macrophages but do not polarize towards M1/M2 subtypes and lack CD86 and MHC-II expression. Bone marrow derived DCs polarize towards DC1 subtype but do not upregulate CD86 and MHC-II. In response to strong control activation signals, the addition of *Coccidioides* inhibits DC and monocyte activation and differentiation. In vivo *Coccidioides* infection induces DC1/DC2 lung responses but no monocyte frequency changes in peripheral blood. White blood cell count increased one day post infection then dropped significantly seven days post infection. Neutrophil count dropped one day post infection then returned to normal by day seven post infection.

CONCLUSION: Our data suggest that *Coccidioides* enhances monocyte differentiation but blocks polarity at the M0 stage and inhibits macrophage activation. DCs polarize in vitro but lack upregulation of activation/maturation markers. In vivo responses to *Coccidioides* shows both DC1 and DC2 presence, signaling a productive immune response in the lung. Ongoing in vivo experiments will assess monocyte differentiation patterns and early immune interaction within the lung microenvironment following infection and the impact of these signals on adaptive immunity. Funded by University of California Valley Fever Research Funding Opportunity and University of California Multicampus Research Programs and Initiatives.

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A Phase IIa Trial Study to Assess Safety and Efficacy of Interferon Gamma in the Treatment of Severe Pulmonary or Nonmeningeal Disseminated Coccidioidomycosis

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Abstract

INTRODUCTION: Disseminated Coccidioidomycosis (DCM) occurs in estimated 1-2% of cases annually, and there are no specific treatments to rescue these patients from high morbidity and mortality. Because defects in robust interferon-gamma-mediated (IFN-γ) Type-1 immune responses result in severe disease, treatment of patients with disseminated disease with systemic IFN-γ has been attempted and has shown promise. Here we report plans to treat patients with DCM with systemic IFN-γ therapy in an upcoming clinical trial.

METHODS: A phase 2A clinical trial will explore whether IFN-γ administered three times weekly in combination with standard antifungal therapies improves clinical outcomes in severe pulmonary and/or non-meningeal disseminated coccidioidomycosis as compared to standard therapy alone. Assessments of efficacy will include reduction in complement fixation titer, C-reactive protein, and Th1/Th2 responses. Exploratory objectives will further understand the impact of type-2 immunity on the disease course of severe coccidioidomycosis and the effect of IFN-γ therapy on the same.

RESULTS: We expect to identify biomarkers to determine which patients would most benefit from this treatment. We expect to see an increase in the proportion of Th1 cells increased after treatment with IFN-γ, further supporting the idea that restoration of the balance between types 1 and 2 immunity is associated with improved outcomes.

CONCLUSION: Introduction of a new phase 2A clinical trial will further examine the role of IFN-γ as a therapeutic element in patients with Disseminated Coccidioidomycosis. This trial will be conducted by Dr. Maria Garcia-Lloret with support from Horizon Pharmaceuticals.

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Peritoneal coccidioidomycosis in a pediatric patient

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Abstract

Introduction: Chronic peritonitis is an unusual extrapulmonary manifestation of coccidioidomycosis that is challenging to diagnose and manage due to its propensity for relapse. It is even more unusual to diagnose peritoneal coccidioidomycosis in the pediatric population.

Methods: We present the case of a previously healthy 5-year-old Filipino female child in Florida who was diagnosed with peritoneal coccidioidomycosis. After eight months of unintentional weight loss and five months of worsening abdominal distention at home, the patient presented to medical care. Imaging revealed significant abdominal ascites and nodularities throughout the peritoneum and lining the surfaces of the intra-abdominal organs. The peritoneal fluid demonstrated a lymphocytic pleocytosis and extensive workup for infections including *Mycobacterium tuberculosis* and parasitic infections were not revealing. CA125 levels were elevated, but peritoneal adenosine deaminase was within normal limits. A biopsy of the affected tissue was later performed and diffuse granulomas surrounding spherules were visualized that were positive on GMS and PAS staining, concerning for Coccidioidomycosis. Exposure history revealed that she was born and raised in California and had traveled throughout the Southwest before moving to Florida one year prior to presentation. Complement fixation titers were significantly elevated at ≥ 1:512 and immunodiffusion titers were positive. A Coccidioides PCR was sent from the tissue to the Mayo clinic and was positive, and fungal cultures from the tissue grew *Coccidioides immitis/posadasii.* Immunologic workup was reassuring. The patient was started on oral Fluconazole with rapid resolution of her symptoms.

Results: Involvement of the peritoneum in Coccidioidomycosis is extremely rare. Individuals of Filipino descent are at increased risk for disseminated Coccidioides. In peritoneal coccidioidomycosis, abdominal distention due to ascites is the most common presentation, and the peritoneal fluid is typically exudative with an increased white blood cell count. Imaging may reveal peritoneal deposits which can mimic other infections and malignancy. Two cases in literature also presented with increased CA125 levels, as in our case. Diagnosis can be based on histopathological demonstration of fungal structures, cultures, antibody testing, antigen detection and/or PCR. Treatment guidelines suggest azole therapy for nonmeningeal disseminated coccidioidomycosis with at least 6–12 months of treatment for extrapulmonary coccidioidal soft tissue infection, though some experts recommend extending therapy to 3 years to minimize the risk of relapse.

Conclusion: Peritoneal coccidioidomycosis is an extremely uncommon condition and it is even more rare in the pediatric population, but should be considered in those in the appropriate clinical settings, particularly if they have history to suggest exposure to regions where this fungus is endemic.

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Inflammasome dependent and independent pathways in Coccidioidomycosis

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Abstract

INTRODUCTION: Coccidioidomycosis is an invasive fungal disease presenting symptoms that range from asymptomatic or mild, to isolated pneumonia, to disseminated disease involving CNS, skin, and bone. This variation may be in part due to distinct differences in host innate immune responses. The primary goal of this study was to explore the role of IL-1 cytokine responses using *ex vivo* assays in fresh whole blood or adherent monocytes to measure and compare relevant cytokine responses among affected subjects (disseminated coccidiomycosis - DCM or uncomplicated valley fever - UVF) and healthy controls (HC).

METHODS: Blood samples from over 70 donors at VFI (34 UVF, 42 DCM) and 8 HC at UCSD were collected with informed consent according to IRB approved protocols. Adherent monocytes (AM) or whole blood (WB) cells were stimulated with either T27K (heat killed *Coccidioides* spherules), Curdlan (β-glucan that activates Dectin -1), or a combination of LPS+ATP (NLRP3 specific stimuli). The NLRP3 specific inhibitor MCC950 (1 μM) was also tested to further evaluate NLRP3-inflammasome dependent responses. Plasma from WB assays (4 hr post-stimulation) or cellular supernatants from AM (16 hr post-stimulation) were collected to determine relative cytokine release between the donor groups. IL-1β, IL-1α, and TNFα levels were measured using ELISA.

RESULTS: T27K elicited a greater IL-1β response in WB from DCM than that of HC but no difference in IL-1α or TNFα, whereas Curdlan stimulated significantly less IL-1α release in WB and AM from both *Coccidioides* affected groups (DCM and UVF) compared to HC. IL-1β and TNFα levels from WB and AM were comparable between all groups with LPS+ATP. However, we observed a significant reduction in IL-1α release in WB from DCM compared to other groups following treatment with LPS+ATP. Inhibition of IL-1β release in AM from affected patients was observed following *in vitro* exposure to MCC950 prior to Curdlan or LPS+ATP but no effect was observed in T27K stimulated samples.

CONCLUSION: Host response to *Coccidioides* have apparent NLRP3 inflammasome dependent and independent pathways leading to IL-1α and IL-1β release. Preliminary data suggests that DCM patients may have novel dysregulated NLRP3 inflammasome mediated responses leading to inadequate IL-1α production, however the exact mechanisms driving its release and the cellular sources are still unclear and merits further investigation.

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Deadly Recombination: Elucidating the Secret Sex Life of *Coccidioides*

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Abstract

Introduction:

Valley Fever is a type of pneumonia caused by inhalation of spores produced by fungi of the genus *Coccidioides*. Infections with these fungi can range from asymptomatic to lethal pneumonia, meningitis or osteomyelitis. One potential explanation for this variation in symptoms may be due to genetic variability mediated by sexual recombination within the fungus. While a case for sexual recombination in these fungi has been hinted at through sequencing of the mating type idiomorphs (MAT1-1 and MAT1-2), previously, no sexual stage of this fungus has ever been visualized under laboratory conditions.

Materials and Methods:

Mating types were established through sequencing the MAT idiomorphs in fungal isolates. Potentially compatible fungi were plated on different media types shown to be conducive to the mating process in other fungi. Fungi were selected from the first round of selection based on microscopic images indicative of the mating process and placed under harem mating conditions. Plates were screened for visible gymnothecia which were harvested and suspended in lactophenol blue and imaged under a Leica DMI3000B.

Results:

Evidence of sexual structures involved in genetic recombination were directly visible under the microscope. These structures included gymnothecia generated by coiled hyphae from compatible mating types, hyphal fusion, and the formation of vase-like vessels believed to be asci. Within asci, visible round spores were visible and are believed to be ascospores (Figure 1).

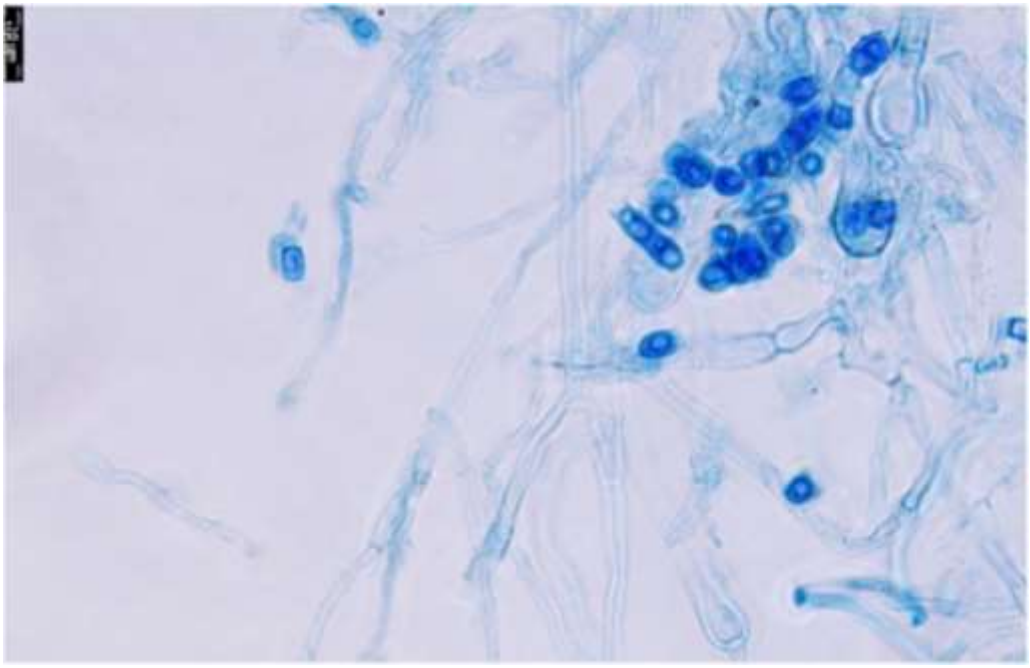

Figure 1. Mating structures of *Coccidioides posadasii*. Mating structures such as the vase-like vessels called asci which contain meisopores (ascospores) and hyphal fusion are visible.

Conclusion:

Until now, it was widely believed that *Coccidioides* spp. only maintained asexual cycles both within the environmental, saprobic stage as well as within in the host, parasitic stage. However, this recent experimental result of the production of sexual spores indicates that a sexual cycle may exist within the environment, saprobic stage. What role this cycle plays in the ecology, evolution, and ability of this organism to cause disease may transform our understanding of this organism and increase our understanding of the wide range of symptoms associated with infections.

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Incorporating Genetic Ancestry as a Biomarker for Disseminated Coccidioidomycosis (DCM)

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Abstract

Introduction: For the past 80 years, epidemiological studies have demonstrated that certain specific racial and ethnic groups were more likely to progress towards severe disseminated Coccidioidomycosis (DCM). While many of these outcomes have been observed over years, the underlying mechanistic and genetic pathogenesis underlying these epidemiological observations have not been fully evaluated. We will explore two major hypotheses: that common variants that make up ancestry-specific approaches underlie race and ethnicity specific differences in DCM or that rare genetic variants in key immune signaling pathways increase risk of DCM.

Methods: Here we have performed exome and genome sequencing from over 50 patients with either Uncomplicated Valley Fever (UVF) or DCM. DNA and RNA was extracted from whole blood and sent for exome-sequencing and RNA-sequencing, respectively. We use PCA to determine whether their genetic ancestry correlates with their risk of DCD. Exome data was maped to GRCh38 using BWA Aligner. Bam files were processed GATK using standard clinical processes and variants were annotated using VarsSeq. We performed traditional PCA analysis to group individuals into their best fit group based on genetic markers. This is a global metric that uses 1000 genome reference panel to determine belonging to a single group. RNA-sequencing data was mapped using Kallisto and explored differential isoform splicing with Sleuth.

Results: RNA-seq signal from our preliminary analysis (n=15, 7 with UVF and 8 with DCM) demonstrate subtle splicing differences. Using a principal component for these splicing differences, thes differentially spliced genes separate out the UVF vs DCM groups and for these 55 genes, including genes previously implicated in pathogenesis such as CHIT1. Gene-ontology analysis using GOseq shows 37 enriched categories (FDR < 0.10), several of which include neutrophil biological processes and other immune system responses. Our analysis of the exome sequencing preliminarily shows increased DCM risk associated with PC1, which distinguishes increasing African-American genetic ancestry.

Conclusions: Using careful analysis, we are able to explore the relationships between genetic ancestry and risk of DCM. Our approaches can explore with finer granularity ancestry-specific genetic regions that

increase risk for DCM and the underlying gene regulatory mechanisms underlying our clinical observations.

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T helper cell phenotypic differences between Disseminated Coccidioidomycosis and Uncomplicated Valley fever

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Abstract

INTRODUCTION:

Infections with coccidioides fungi are endemic in the Southwestern United States with 1-2% progressing to severe, disseminated coccidioidomycosis (DCM), defined as spread beyond the lungs, often including the central nervous system, skin, and/or musculoskeletal systems. Robust type 1 immunity, mediated by interferon-gamma (IFN-γ), plays a critical role in resolution of disseminated disease. It has been previously reported that an imbalance in type 1 versus type 2 immunity may confer susceptibility to severe DCM. T-helper 17 (Th17) cells also play important roles in immunity against yeast and fungi. Here we sought to identify how generalizable these findings are by comparing a large group of patients with DCM and uncomplicated Valley fever (UVF) for their T helper phenotypes.

METHODS:

Blood samples from 38 donors at Valley Fever Institute (7 UVF, 31 DCM) and 11 healthy controls at UCLA were collected with informed consent according to IRB-approved protocols. T cells were analyzed by flow cytometry via staining for Th1 (CCR6-CCR4-CXCR3+), Th2 (CCR6-CCR4+CXCR3-), and Th17 (CCR6+CCR4+CXCR3-) markers and for their production of intracellular cytokines upon stimulation.

RESULTS:

We identified subsets of patients with abnormal T helper cell phenotypes. We found that 30-40% of subjects with DCM demonstrated an excess in Th2 cells as compared with those with history of UVF. We also identified a subset of patients with low Th17 cells.

CONCLUSION:

These findings show that T helper cell dysfunction compromises immunity in a large subset of subjects with DCM. Excessive Th2 activity, or deficient Th1 activity, are potentially therapeutically actionable, as we previously published. The underlying causes for these skewed T cell responses is not yet clear and is the subject of ongoing genomic and transcriptomic analyses.

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Co^3: CoVID, Cocci, and Co-infection.

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Abstract

INTRODUCTION:

Both COVID-19 and coccidioidomycosis present with fever and respiratory symptoms; as such, simultaneous testing for COVID and coccidioidomycosis was commonly performed in Arizona within Banner Health. Many of the patients found to be positive for COVID by PCR were also positive for Coccidioidomycosis by EIA.

METHODS:

After IRB approval, we requested data from Banner Health to study the dynamics of SARS-CoV-2 and Coccidioides co-infection. All patients with a SARS-CoV-2 PCR test and a coccidioidal serology done within 10 days of each other in calendar year 2020 through January, 2021 were enumerated. Separately, all patients with both a positive SARS-CoV-2 and a positive coccidioidal serology (EIA, immunodiffusion, or CF) test before January 1, 2021 were identified and sorted by the magnitude of the date difference for first positive tests (SARS-CoV-2 minus Cocci range: -366 to 422 days). Chart review is underway for this series of patients to analyze the clinical findings of those with confirmed newly identified coccidioidomycosis proximately to COVID, beginning with those whose first test date difference is smallest (same day testing).

RESULTS:

In 17,974 patients, there was a significant inverse relationship between SARS-CoV-2 PCR positivity and coccidioidal serology (Chi-squared, $p<0.001$). Most (58.0%) were positive for neither, 31.8% were SARS-CoV-2 alone, 8.0% for coccidioidal serology alone, and 2.3% were positive for both. Hence, testing for both SARS-CoV-2 and coccidioidal antibodies increased the rate of a specific diagnosis by 23%, from 34.1% (total SARS-CoV-2 PCR positive) to 42.1% (either positive). In other studies, a similar percentage (16.9%) was found in 302 Banner Health urgent care patients in 2019 serologically tested for coccidioidomycosis.

Of those found to have both SARS-CoV-2 and coccidioidal tests positive, 204 were positive within one day of each other. Based on a sampling of these patients and excluding those with coccidioidomycosis in the distant past, younger patients appeared to more likely have a coccidioidal clinical diagnosis compared to older patients presenting with a clinical syndrome consistent with COVID.

CONCLUSION:

Within our Coccidioides-endemic region, also testing for coccidioidal antibodies in patients suspected of COVID produced 16.6% more specific diagnoses, with a commensurate reduction in the need for prescribing empiric antibacterial drugs. The dual positive patient population could represent individuals with true co-infection, but other possibilities such as reactivation of past disease need further investigation.

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Development of a quantitative antigen assay to detect coccidioidal chitinase-1 (CTS1) in human serum

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Abstract

Introduction. Coccidioidomycosis is currently diagnosed with a network of assays that rely on the patient's ability to mount an immune response to the fungus (antibody-based diagnostics), making identification of this infection challenging. Here we present an antigen-based diagnostic that detects and quantifies coccidioidal CTS1 (complement fixation, (CF) antigen) to allow for rapid and accurate diagnosis.

Methods. An inhibition-based enzyme-linked immunoassay (ELISA) was developed that utilizes a monoclonal antibody specific for coccidioidal CTS1. CTS1 was quantified in commercial antigen preparations using the inhibition ELISA using recombinant CTS1 as a known standard. Sera from 238 patients were tested, which included 45 patients (18.9%) with a positive complement-fixation titer, and 38 additional patients (16%) with at least one positive *Coccidioides*-related serodiagnostic.

Results. If we consider a minimum of one positive serodiagnostic the basis of a true positive for coccidioidomycosis, the sensitivity and specificity of the CTS1 inhibition ELISA was 72.3% and 94.2% respectively. The quantity of CTS1 in different commercial antigen preparations from different suppliers varied. CTS1 antigenemia was detected in 72.3% of patients with at least one positive serology result. The quantity of CTS1 detected in patients with low- and high-titer antibodies against *Coccidioides* was statistically significant.

Conclusions. Since the CTS1 inhibition ELISA described and evaluated in this report does not depend on the host immune response, it has potential as a diagnostic tool in clinical laboratories to aid in diagnosis and disease monitoring of coccidioidomycosis.

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HEPATIC ABSCESS IN A PATIENT WITH DISSEMINATED COCCIDIOIDOMYCOSIS: A CASE REPORT

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Abstract

Introduction:

Forty percent of patients with coccidioidomycosis develop a self-limited respiratory infection; however, 5% of these individuals develop extra pulmonary dissemination. Dissemination to liver frequency is unknown and is only limited to a few published case reports presenting as granulomatous hepatitis. Here is a case of disseminated Coccidioidomycosis, who presented for symptoms of recurrent pneumonia and was found to have a coccidiosis hepatic abscess, a rare condition.

Methods:

Approval was obtained from the Institutional Review of Board of Kern Medical with IRB # 20021. A retrospective review of patient record was performed. A literature search was conducted on PubMed, Google Scholar, Infectious Disease society of America (IDSA)'s Clinical infectious disease Journal database. The following search terms were applied: Coccidioidomycosis, Disseminated coccidioidomycosis, Hepatic abscess, Coccidioidomycosis involving liver

Results:

A 50-year-old male with type 1 Diabetes mellitus, end-stage renal disease (ESRD) on hemodialysis. He was originally diagnosed with pulmonary coccidioidomycosis in 2005 and in 2007 was found to have disseminated tibial osseous and coccidioidomycosis meningitis. Patient was previously treated with Amphotericin B that was transitioned to voriconazole.

In 2020, Patient was experiencing subjective intermittent fevers, cough, and dyspnea for 2 months and received 2 courses of treatment for community acquired pneumonia (CAP) without improvement. He presented to our Emergency Department (ED) with low grade fever and leukocytosis of 15.5x10^3/mcL with neutrophilia. Liver tests showed alkaline phosphatase of 219, aspartate aminotransferase (AST) of 99, alanine aminotransferase (ALT) of 45, albumin 1.9, and total protein 6.5. Coccidioides immunodiffusion IgG and IgM assays were both reactive with complement fixation of ≥1:512. Chest CT scan revealed diffuse patchy right lung airspace disease with ground glass opacities. CT chest also showed an incidental collection in the liver. As a result, then CT abdomen was obtained and demonstrated a rim enhancing hepatic septate collection measuring 7.3 x 6.8 x 5.4 cm. Patient underwent an ultrasound guided percutaneous drainage and sampling of the hepatic collection with placement of a percutaneous drain. PAS staining of the fluid showed multiple spherules with endosporulation resembling coccidioidomycosis as shown in figure 1. Fungal culture eventually grew

and was sent to reference fungal laboratory (University of Texas at San Antonio) where growth of C. immitis was confirmed. Antifungal susceptibility testing at the same reference fungal laboratory for Amphotericin B, Fluconazole, Itraconazole, and Voriconazole MICs were ≤0.03, 8, 0.06, and 0.125 mcg/mL, respectively.

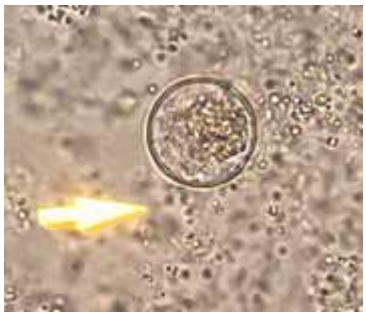

Figure 1: KOH wet mount showing spherules.

Parental treatment with liposomal amphotericin B was discussed with the patient but he deferred. Therefore, he was switched from oral voriconazole to oral Isavuconazonium 372mg daily and discharged with close follow up with the infectious disease clinic.

Conclusions:

This case demonstrates a rare manifestation of disseminated coccidioidomycosis to the liver, hepatic abscess. As per our literature search first case to be reported. This case aids in providing details of a rare condition encountered with diagnostic and therapeutic approach.

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RAPIDLY PROGRESSIVE COCCIDIOIDES MENINGITIS IN A PATIENT WITH RHEUMATOID ARTHRITIS ON HUMAN INTERLEUKIN-6 (IL-6) RECEPTOR ANTAGONIST (SARILUMAB)

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Abstract

Introduction. The risk of severe coccidioidomycosis in immunosuppressed patients can increase up to 150-fold. An ongoing clinical question is the safety of biologic response modifiers (BRM) in coccidioidomycosis endemic regions. Here we present a case of severe, rapidly progressive *Coccidioides* meningitis in a patient who was treated with an anti-IL-6 receptor monoclonal antibody, sarilumab, for severe rheumatoid arthritis.

Case Description. A 66-year-old Hispanic woman, born in Mexico, moved to Tucson, Arizona 30 years ago, with a medical history significant for hypertension, hyperlipidemia, anxiety, and rheumatoid arthritis (RA). She received etanercept (a TNF inhibitor) for > 4 years with poor response. This was replaced with sarilumab monotherapy in November 2019. In October, 2020 she developed mild SARS-CoV-2 infection which resolved without treatment or a chest X-ray. Subsequently, she developed severe headaches, exacerbated by bright lights and accompanied by nausea and vomiting. Her symptoms progressed with poor appetite and a 20 pound weight loss. Initial brain MRI on November 23 showed only diffuse mild cerebral volume loss. Despite symptomatic treatment, she showed minimal improvement. By January 2021, she had significant gait disturbance, dysarthria and refusal of oral intake, including medication. In February, 2021, she was referred for hospitalization. Computed tomography (CT) imaging of brain showed diffuse ventriculomegaly compatible with acute communicating hydrocephalus. Urgent Neurosurgerical evaluation prompted external ventricular device (EVD) placement. Brain MRI now showed severe nodular leptomeningeal enhancement at the surfaces of the supratentorial and infratentorial brain into the upper cervical spinal cord. Chest CT showed multiple scattered solid pulmonary nodules measuring up to 6 mm. CSF studies (from lumbar puncture) showed WBC of 60/mm3, 75% lymphocytes, glucose of 25 mg/dl and protein of 115.7 mg/dl. CSF cultures, Cryptococcus antigen, MTB PCR were negative. CSF *Coccidioides* EIA IgG and *Coccidioides* Ag were positive. Serum *Coccidioides* EIA, IgM and IgG, as well as IMDF IgG were positive. Serum *Coccidioides* complement fixation titer was <1:2. She was started on fluconazole 400 mg daily. Mental status improved. Repeat CSF studies (from EVD) in a few days showed decreased cell count (WBC 5/mm3), decreased protein 58 mg/dl, and normal glucose 64 mg/dl. A permanent ventriculo-peritoneal shunt (VP) was placed and she entered rehabilitation.

Discussion. Immunosuppression, including BRM treatment, can either worsen the consequences of newly acquired coccidioidal infection or allow a prior, latent coccidioidal infection to reactivate. Since this patient has many years of endemic residence prior to her current illness, it is not clear which occurred. The observation that the patient had no problems with anti-TNF therapy raises the possibility that this represented a new infection, rather than a recurrence of a previously stable infection. Although the diagnosis of SARS-CoV-2 pneumonia shortly before the onset of her neurologic symptoms could be a confounding factor, this patient's rapidly progressive *Coccidioides* meningitis very possibly may be due to dysregulation of the IL-6 axis by sarilumab. This case suggests that the IL-6 cytokine axis and its signals may be important for the control of *Coccidioides.*

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TRANSCRIPTOMICS OF THE COCCIDIOIDOMYCOSIS VACCINE STRAIN *Δcps1* REVEALS INSIGHTS INTO *CPS1* FUNCTION

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Abstract

INTRODUCTION:

A live-attenuated coccidioidomycosis canine vaccine is based on a *Coccidioides posadasii* Silveira mutant with a *CPS1* gene deletion. The avirulent *Δcps1* fails to produce mature spherules *in vitro* and *in vivo,* although has only minor somatic growth defects. The *Δcps1* spherules undergo plasmolysis without differentiating into endospores. Sequence analysis of *CPS1* suggests it encodes a membrane receptor protein that has catalytic functions and regulates downstream gene expression, but the details of its function are unknown. To gain insights into Cps1 function, the transcriptomes of Silveira and *Δcps1* during mycelial and spherule growth *in vitro* were compared.

METHODS:

To perform RNA-seq, RNA was harvested from 24 hr and 48 hr spherules, and 48 hr mycelia of Silveira and *Δcps1*. Spherules were grown in RPMI with 20% CO_2 and mycelia were grown in 2xGYE liquid media. Three biological replicates were prepared for each condition. Illumina reads were mapped to the *C. immitis* RS genome and analyzed for differentially expressed genes.

RESULTS:

RNA-seq data revealed 1446 differentially expressed genes at one or more time points, 24h, 48h spherules, or 48h mycelium, based on at least a two-fold change. Clustering analysis identified 264 sets of co-expressed genes. *CPS1* clusters with other genes not expressed in the mutant, and contained two key genes in acetyl-CoA production. Other acetyl-CoA pathway genes are also downregulated in *Δcps1.* Biochemical analysis revealed a significant reduction of acetyl-CoA in *Δcps1*. We hypothesized this impacts membrane integrity; supporting this, *Δcps1* is hypersensitive to SDS. This defect may activate the cell wall integrity pathway; expression data and sensitivity assays to calcofluor white and congo red support this. Further, expression data indicates activation of the ER stress response in *Δcps1* possibly due to reduced membrane integrity and increased expression of secreted proteins.

CONCLUSION:

The large number of differentially expressed genes between *Δcps1* and Silveira supports Cps1 as a regulatory protein and the reduced acetyl-CoA levels indicate a key defect in cellular metabolism. Reduced membrane and cell wall integrity of spherules may be responsible for premature spherule plasmolysis *in vivo* and *in vitro*. Understanding the biological consequences of the *CPS1* deletion are key to understanding the safety of this live-attenuated vaccine.