1 POSTER

COCCIDIOIDOMYCOSIS AND ASPERGILLOSIS IN A PATIENT WITH PULMONARY TUBERCULOSIS SEQUELAE

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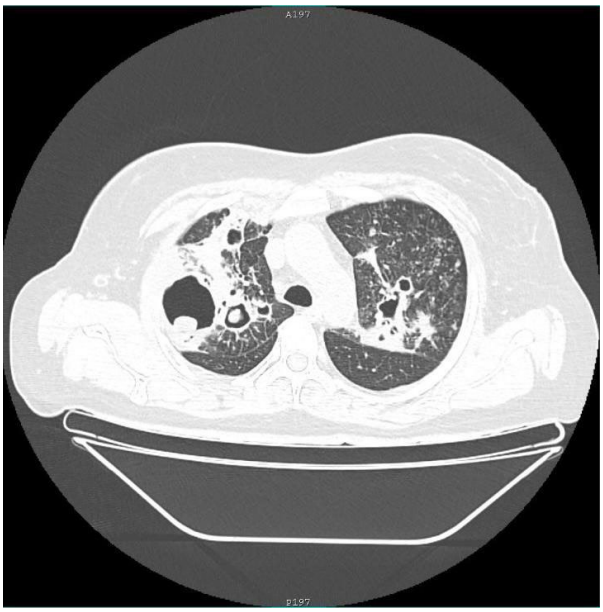
Abstract

Introduction: Coccidioidomycosis is exclusive to the American continent, being endemic in the southwestern United States and northwestern Mexico. Aspergillosis is an opportunistic mycosis caused by the saprophytic soil fungus *Aspergillus* spp. The main risk factors that favor the presence of these mycoses are structural lung damage and immunocompromised state.

Clinical case: We present a case of coinfection by *Aspergillus* spp. and *Coccidioides* spp. in a patient with a history of pulmonary tuberculosis and diabetes mellitus. Sputum examination reported the simultaneous isolation of *Coccidioides* spp. and *Aspergillus* spp.

Conclusions: In patients with respiratory symptoms and a history of tuberculosis and fibrocavitary sequelae, it is essential to rule out initially a tuberculosis relapse, but at the same time, investigate the presence of endemic and opportunistic fungi.



2 POSTER

REBOUNDING COCCIDIOIDAL COMPLEMENT FIXATION SEROLOGIC TITERS AFTER DISCONTINUATION OF ANTI-FUNGAL TREATMENT: AN OBSERVATIONAL STUDY.

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Abstract

Introduction: Primary pulmonary coccidioidomycosis is the most common presentation of coccidioidomycosis. While most such infections treated with antifungal resolve over time, occasionally a rise in complement fixation (CF) titers is observed after the discontinuation of antifungal treatment. We aimed to describe the outcomes of infection in this subset of patients.

Methods: This study was approved by Mayo Clinic Institutional Review Board. We identified patients with primary pulmonary coccidioidomycosis by searching electronic health records from 1/1/2000 to 12/31/2020 using International Classifications of Diseases ninth revision (ICD-9 114.0 and 114.5) and tenth revision (ICD-10 B38.0 and B38.2) for Acute Pulmonary Coccidioidomycosis and Primary Pulmonary Coccidioidomycosis, respectively. We included immunocompetent, non-pregnant adult patients with probable or proven pulmonary coccidioidomycosis, who received antifungal treatment, and whose complement fixation (CF) titer increased by ≥ 2 dilutions following discontinuation of antifungal treatment.

Results: We initially identified 1292 adult patients with probable or proven pulmonary coccidioidomycosis, 56 (4.3%) of whom experienced a CF titer increase of ≥2 dilutions following treatment discontinuation. Of the 56, 70% were white males with a median age of 70 years, and median rebounded CF titer of 1:16 (range 1:4 – 1:256). Among the 56, 28 (50%) patients had symptoms in addition to the rise in CF titer ("relapsed infection") and 28 (50%) were asymptomatic. The median time to serologic rebound was 3-4 months. Median duration of follow up after stopping initial treatment was 30 months (range 12 – 139). Antifungal treatment was reinitiated for most patients with relapsed infection, and in half of the 28 asymptomatic patients. Coccidioidal meningitis manifested in 3 (10.7%) of 28 patients with relapsed infection, and skeletal infection manifested 4 years following a second course of antifungal treatment in one (3.7%) of the asymptomatic 28.

Conclusion: In patients whose primary pulmonary coccidioidomycosis was treated with an azole antifungal agent and discontinued, a subsequent elevation of CF titer was infrequently associated with disseminated infection and seen more frequently in the patients whose elevated titers were accompanied by symptoms. This niche patient group requires prolonged follow up, but not necessarily further antifungal treatment.

3 POSTER

FROM DUST TO COSTS: THE IMPACT OF COCCIDIOIDOMYCOSIS IN PUBLIC HEALTH SERVICES

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Abstract

ABSTRACT

INTRODUCTION: Coccidioidomycosis (CM) is a systemic disease caused by Coccidioides immitis and Coccidioides posadasii, inhabiting dry and hot regions in América, mainly the U.S.A.-México border states. The diagnosis is usually challenging because of clinical signs' unspecificity, and it is estimated that 25% of symptomatic cases are genuinely detected in the U.S.A.

METHODS: This is an exhaustive review of articles published from 2002 to November 15, 2022. Every original article containing significant CM-related healthcare costs results and written in English was considered.

RESULTS: There is a direct correlation between time for diagnosis and healthcare-related costs, up to $915 (USD)/patient when diagnosed 120 – 180 days later. Nearly half of the patients are detected in >30 days after first presentation. A quarter of sick people attend repetitively for attention, and ~40% of uncomplicated respiratory patients are hospitalized in the first year with a length-stay of ~ 7 days. Loss of productivity in CM occurs because symptoms affect the host's availability to work for ~14 days. The therapy for CM usually lasts ~6 months, but this can take longer for life in severe/disseminated disease. A low mortality rate of 0.59/1,000,000 persons-years, but a rising incidence has been observed in the U.S.A. since 2014 for CM.

CONCLUSION: delay in diagnosis and underdiagnosis; frequent ER visits and hospitalization; long-term therapy and prophylaxis; inability, absenteeism, and loss of wages for premature death, and an increasing incidence with relatively low mortality are factors contributing to a high economic burden of CM and a severe problem of public health for both countries.

4 ORAL

ISAVUCONAZOLE IN THE TREATMENT OF CHRONIC FORMS OF COCCIDIOIDOMYCOSIS

Arash Heidari[1], Rupam Sharma[1], Qusai Shakir[1], Madiha Shah[2], Josh Clement[2], Monica Donnelly[2], Trina Reynolds[3], Kate Trigg[2], Jeff Jolliff[1], Rasha Kuran[1], Royce Johnson[1], George Thompson[2]
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Abstract

INTRODUCTION: Coccidioidomycosis is a fungal infection with a range of clinical manifestations. Currently used antifungal agents exhibit variable efficacy and toxicity profiles necessitating evaluation of additional therapeutic options.

METHODS: Patients with coccidioidomycosis who received isavuconazole were identified by cross-indexing ICD-9 and ICD-10 codes from patients and data abstracted. Responses to isavuconazole therapy were measured using a modified Mycoses Study Group Coccidioidomycosis Scoring system as described previously.

RESULTS: Eighty-two patients met the criteria for inclusion. Over half of the patients exhibited pulmonary involvement 45/82 (55%), although meningitis 32/82 (39%), bone and joint disease 14/82 (17%), and skin/soft tissue infection 7/82 (9%) were also seen. The majority of patients experienced a decrease in their MSG score following initiation of isavuconazole therapy (median MSG score change across all patient groups $7 \rightarrow 2$, $p < 0.0001$). Overall improvement was noted in 58/82 (71%) patients, while no change was observed in 19/82 (23%) and 5/82 (6%) who were unresponsive to antifungal changes.

CONCLUSION: Isavuconazole demonstrated efficacy in the majority of patients treated, with failures observed only in a subgroup of patients with coccidioidal meningitis.

5 POSTER

PREVALENCE OF COCCIDIOIDOMYCOSIS IN PRIMARY IMMUNODEFICIENCY: DATA FROM THE USIDNET REGISTRY

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Abstract

Introduction: Coccidioidomycosis is a fungal infection endemic to the Southwestern United States, caused by the dimorphic fungi species Coccidioides immitis and Coccidioides posadasii. Most commonly, coccidioidomycosis causes a self-limited mild respiratory illness. However, in certain populations such as immunocompromised patients, may cause disseminated disease, and may be fatal. We aimed to assess the prevalence of coccidioidomycosis in primary immunodeficiency (PID) using the USIDNET registry.

Methods: We queried the USIDNET database on 17 March 2022 requesting demographic data on PID patients with a diagnosis of coccidiomycosis.

Results: We identified ten patients. Four patients (40%) identified as male. Nine patients identified as Caucasian. The median age of diagnosis with PID was 31.2 years. The most frequently reported PID in the cohort was common variable immunodeficiency (CVID) in five patients followed by chronic granulomatous disease (CGD) in two patients. Six patients were diagnosed with coccidioidomycosis prior to their PID diagnosis. of the cohort, three developed disseminated disease. Two patients developed pulmonary complications and one developed hydrocephalus. Four patients received antifungal prophylaxis. No patients underwent gene therapy or stem cell transplantation. Two patients, one with a STAT1 gain-of-function and another with an autosomal recessive pathogenic NCF1, received interferon gamma therapy.

Conclusions: This is the first report on the prevalence of coccidioidomycosis from the USIDNET registry. The prevalence was much lower than expected and highlights the likelihood of underreporting of coccidiomycosis in the PID population. When evaluating a patient with disseminated coccidiomycosis, an immunodeficiency evaluation may be beneficial to uncover potential undiagnosed immune defects.

6 POSTER

DETERMINING THE PREVALENCE OF *COCCIDIOIDES* SPP. IN SEMI-ARID SOILS OF TEXAS

Christopher Cobos[1], Daniel Kollath[2], Bridget Barker[2], Joseph Burke[1], Riley Babcock[1], Katie Lewis[1]
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Abstract

Introduction: The ecological niche and climatic parameters ideal for *Coccidioides* spp. prevalence is not completely elucidated and the published endemic range is most likely outdated. Texas has historically been included in this range; however, few studies have focused on identifying soil and environmental bioaerosol samples across the state for the fungal pathogen.

Methods: Seven counties across the Texas Southern High Plains were soil sampled for molecular identification of *Coccidioides* spp. Soil sampling was limited to only terrestrial burrows caused by animal activity. Topsoil at burrow entrance and within approximately 6 in. - 2 ft. (depending on burrow size and depth) inside of the burrow were pooled and collected in two separate 50 mL centrifuge tubes. Samples were immediately capped and kept at 4 degree Celsius for storage. All samples were lyophilized for 24 hours in a Labconco FreeZone 2.5 Liter Benchtop Freeze Dry System. Dried samples were stored at room temperature for molecular PCR testing.

Results: 125 separate soil samples were tested from seven Texas counties. *Coccidioides* spp. was not positively identified via PCR confirmation.

Conclusion: Understanding the geographic distribution and natural habitat factors relevant to the growth and prevalence of *Coccidioides* spp. in the soil will help in elucidating these parameters as well as positively affecting the epidemiological outcome of Valley Fever. So far, no current samples have been positively identified for *Coccidioides* spp. Environmental soil sampling is continuing and expanding in year two of the project to include predominant southern counties across the Texas Southern High Plains and Trans Pecos region. Aerosol dust collection will also be implemented in the future for molecular confirmation of the *Coccidioides* spp. via bioaerosol samples.

7 POSTER

WIND EROSION ON THE TEXAS HIGH PLAINS: PAST PROBLEMS, FUTURE OPPORTUNITIES

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Abstract

INTRODUCTION:

Wind erosion is synonymous with the Texas High Plains, and modern soil conservation owes its beginning to the Dust Bowl of the region. Understanding the history of the Dust Bowl, efforts to reduce wind erosion, and governmental policies that influence conservation adoption can help guide research priorities.

METHODS:

A review of 100 years of soil erosion research was conducted, and relevant results will be presented.

RESULTS:

Results include a historical analysis of wind erosion on the Texas High Plains and its impact on the people.

CONCLUSIONS:

Wind erosion has significantly impacted the Texas High Plain and will continue to impact the region despite reductions in erodibility.

8 POSTER

CASE REPORT: PRIMARY CUTANEOUS COCCIDIOIDOMYCOSIS BY *COCCIDIOIDES POSADASII*

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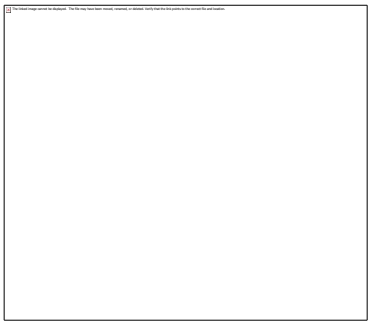
Abstract

Introduction: Coccidioidomycosis is a systemic fungal infection caused by two dimorphic ascomycete fungi, the primary cutaneous form is presented in the 2 – 3% of the cases.

Methods: We realize direct mycology examination with 10% potassium hydroxide, as well a biopsy and the culture in Saboraud agar, from which we performed PCR. Literature search was conducted on Google Scholar, PubMed.

Results: A 44-year-old man from Sinaloa, Mexico that present a nodule in the left elbow, 15 days previous he had a trauma with the branch from a tree in the same region. He was prescribed trimetroprim with sulfametoxazole before presentation to our center with the presumptive diagnosis of mycetoma. He subsequently developed a plaque in the proximal left forearm and elbow with multiples nodules, secretion, and scabs. When he arrived to our center we realize direct mycology examination with potassium hydroxide of the secretion of a nodule that showed fungal spherules; the biopsy of the lesion revealed granulomatous dermatosis and fungal spherules with endospores in the interior; a cottony whitish colony grew on the culture. With these findings he was diagnosed with coccidioidomycosis and was prescribed with itraconazole 200 mg daily. The blood analysis, pulmonary and bone X-ray were normal. A culture sample was sent to the National Institute of Respiratory Diseases (INER) where the species of *Coccidioides posadasii* was identified with PCR and amplification of microsatellites. The patient has been with the same treatment for 8 months without the appearance of new lesions.



Conclusions: The primary cutaneous form of coccidioidomycosis is an unusual presentation, there are only 82 cases in the world from 1929 to 2019.

9 POSTER

A CASE OF COCCIDIOIDAL MENINGITIS TREATED WITH SUBA-ITRACONAZOLE

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Abstract

INTRODUCTION: Itraconazole is an azole antifungal that has been used to successfully treat coccidioidal meningitis (CM), although the use of this agent is limited by highly variable bioavailability due to pH-dependent absorption resulting in interactions with food and other drugs, particularly antacids. Recently, a super bioavailable formulation of itraconazole (SUBA-ITR) that exhibits more reliable absorption, including increased exposure with concomitant proton pump inhibitor (PPI) therapy, has become available for clinical use. To our knowledge, no cases have been published reporting successful treatment of CM with SUBA-ITR. We describe a case of CM complicated by toxicities to multiple azoles, ultimately treated with SUBA-ITR.

METHODS: Retrospective case review after IRB approval

RESULTS: A 25-year-old man with CM diagnosed 2 months prior at another facility presented to the hospital with worsening headache, blurred vision, and general weakness. At initial diagnosis, treatment was started with fluconazole 800 mg daily, with only mild symptom improvement, so the dose was intensified to 1200 mg daily. However, he experienced vomiting attributed to fluconazole, resulting in nonadherence to therapy. At this presentation, primary admission diagnosis was CM/leptomeningitis and arachnoiditis, and he had an initial lumbar puncture with opening pressure 330 mm H_2O, 532 WBC/mcL, glucose 26 mg/dL, and protein 693 mg/dL, with CSF coccidioidomycosis complement fixation (cocci CF) titers at 1:128. Fluconazole was discontinued due to lack of tolerability and he was discharged on isavuconazole for CM. He developed severe transaminitis requiring readmission within 2 weeks, which resolved quickly upon discontinuation of isavuconazole. In an effort to use an azole antifungal with lower potential for hepatotoxicity, itraconazole was started, with improvement in CSF findings while on antifungal therapy. However, he developed severe gastritis requiring chronic PPI therapy from corticosteroid use for arachnoiditis. Due to the major drug-drug interaction between itraconazole and PPIs resulting in decreased itraconazole levels, he was switched to SUBA-ITR (Tolsura) 65 mg twice daily. Most recent follow-up approximately 16 months later revealed consistent improvements in CSF findings (opening pressure 110 mm H_2O, 21 WBC/mcL, glucose 36 mg/dL, protein 125 mg/dL) and cocci CF titer (1:4), and itraconazole levels were consistently within therapeutic range while on pantoprazole 40 mg twice daily.

CONCLUSION: While clinical evidence supports the use of itraconazole for treatment of CM, limited pharmacokinetic or clinical data is available regarding the use of SUBA-ITR for CNS disease. This is the first reported case of CM treated successfully with SUBA-ITR.

10 POSTER

LUNG NODULE PREDICTION TOOL FOR USE IN AREAS ENDEMIC TO COCCIDIOIDOMYCOSIS

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Introduction Lung nodules are common. They may represent lung cancer or may be due to endemic fungi including coccidioidomycosis (cocci). Currently validated lung nodule risk calculators inappropriately classify 84-94% of cocci nodules as intermediate-to-high risk for lung cancer resulting in unnecessary costs and risks. We aimed to develop a better prediction model to differentiate nodules due to cocci from those due to lung cancer in areas endemic to cocci.

Methods

Study design: We identified patients seen in the UCSF Fresno multidisciplinary lung nodule program who were diagnosed with stage I-IIIB lung cancer or cocci between 01/01/2011 and 10/31/2022. Patients were identified using ICD codes. We excluded patients diagnosed with stage IV lung cancer or without an official radiology report in our electronic medical record (EMR). We utilized information from the EMR to develop the clinical decision tool. Patients diagnosed with cocci were categorized as proven (biopsy, culture, or PCR positive), probable (radiographic and clinical stability in the setting of necrotizing granuloma on biopsy or positive cocci serology), or possible (large nodule without an alternative diagnosis with clinical and/or radiographic stability). We conducted chart reviews to collect candidate predictor variables including clinical characteristics (age, sex, smoking pack years, personal history of malignancy, family history of lung cancer, clinical history of chronic lung disease, and occupation/exposures); radiographic findings (diameter, location, density, border characteristics, calcification, cavitation, satellite nodules, multiple nodules, radiographic evidence of chronic lung disease, and mediastinal adenopathy); and serologic test results (immunodiffusion (ID) and complement fixation (CF)). Radiographic information was collected from official radiology reports in our EMR. This study was approved by our institutional review board (IRB# 2022019).

Statistical analysis: Descriptive statistics described the sample characteristics of patients with lung cancer and cocci. Measures of association was tested using chi-square or Fisher's exact test. A random ⅔ sample of the data set was used as the development data set. The remaining ⅓ served as the validation data set. Univariable analyses were performed on the development data set using simple logistic regression. Predictors with $p<0.05$ based on the univariable analysis were included in the development of the multivariable model. Stepwise, backward multivariable logistic regression models were performed, with the final model containing plausible predictors with a p-value <0.05. Goodness of model fit was evaluated using a Hosmer-Lemeshow test. The predictive performance of the integer clinical prediction score in predicting lung cancer vs. cocci was assessed by using the area under the Receiver Operating Characteristic (ROC) curve (AUC) in the validation cohort. Sensitivity and specificity were evaluated at each cut-point with 95% confidence interval (CI) estimated. Prediction models derived using the development data set were validated by means of an assessment of discrimination accuracy in the validation data set. Statistical analyses were performed using Stata (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC).

Results We identified 806 patients who met our inclusion criteria - 566 patients diagnosed with stage I-IIIB lung cancer and 240 patients diagnosed with cocci. Of the patients diagnosed with cocci, 125 (52.1%) had proven cocci, 111 (46.3%) probable, and 4 (1.7%) possible. 150/240 (62.5%) patients with cocci underwent CT-guided or transbronchial biopsy and 70/240 (29.2%) underwent bronchoalveolar lavage (BAL). 164/240 (68.3%) underwent one or both invasive procedures. A random ⅔ sample (n=532) was used as the development data set and the remaining ⅓ (n=274) served as the validation data set.

Univariable analyses of clinical, radiographic, and serologic candidate predictors using the development data set were performed. 8 clinical characteristics (age, sex, pack years, personal history of malignancy, family history of lung cancer in a 1st degree relative, clinical history of chronic lung disease, work in agriculture, and exposure to asbestos), 6 radiographic findings (nodule location, density, border characteristics, cavitation, satellite nodules, and radiographic evidence of chronic lung disease), and both serologic tests (ID and CF) were independent predictors, with $p<0.05$. These

predictors – except for ID and CF, which were excluded due to excess missing data – were used in a stepwise logistic regression analysis to derive the multivariable model seen in table 1. The results of the ROC curve analyses showed good overall discrimination accuracy for both the development (AUC = 0.91; 95% CI, 0.88-0.93) and validation data sets (AUC = 0.90; 95% CI, 0.86-0.94). The optimum cutoff point identified by Youden's test was 6. When patients in the validation data set met ≥ 6 of the following criteria – age < 60, male sex, < 50 pack years, no exposure to asbestos, no ground glass component(s), right middle or left upper lobe, no spiculated/lobulated/irregular borders, cavitation, satellite nodules, no radiographic evidence of chronic lung disease – there was a sensitivity of 0.80 (95% CI, 0.72-0.89) and specificity of 0.90 (95% CI, 0.85-0.94) for diagnosing the nodule as cocci.

Conclusion This is the first validated prediction tool developed to distinguish lung nodules due to cocci from those due to lung cancer. It utilizes standard clinical and radiographic data, and, if further validated, can significantly reduce the costs and complications associated with expensive and invasive studies to diagnose cocci nodules.

Table 1. Multivariable Model

Predictor	**OR of Cocci**	**95% CI**	**p-value**
Age < 60	4.127	[2.125, 8.012]	0.000
Male sex	5.248	[2.576, 10.689]	0.000
Pack years			
0	58.256	[12.026, 282.191]	0.000
1-19	9.135	[1.851, 45.081]	0.007
20-49	5.570	[1.240, 25.017]	0.025
50-79	1	NA	NA
≥80	3.645	[.418, 31.780]	0.242
No exposure to asbestos	3.519	[1.290, 9.599]	0.014
Nodule density			
Solid	29.791	[2.263, 392.254]	0.010
Mixed	1	NA	NA
Ground glass	1	NA	NA
Other (e.g. necrotic, cystic, heterogenous)	50.966	[1.657, 1567.755]	0.025
Nodule location			
Right upper lobe	1.462	[.589, 3.629]	0.413
Right middle lobe	3.961	[1.019, 15.396]	0.047
Right lower lobe	1	NA	NA
Left upper lobe	3.147	[1.177, 8.413]	0.022
Left lower lobe	2.193	[.806, 5.964]	0.124
Border characteristics			
Smooth/well-circumscribed	31.484	[4.930, 201.060]	0.000
Lobulated	2.000	[.598, 6.697]	0.261
Spiculated	1	NA	NA
Other border irregularity (e.g. irregular, ill-defined, indistinct)	2.206	[.641, 7.594]	0.210
None of the above	6.552	[2.572, 16.689]	0.000
Cavitation	5.466	[2.110, 14.161]	0.000
Satellite nodules	23.998	[6.128, 93.981]	0.000
No radiographic evidence of chronic lung disease	3.427	[1.343, 8.744]	0.010

11 ORAL

NOVEL EPIDEMIOLOGICAL TRENDS OF COCCIDIOIDOMYCOSIS IN SOUTH AMERICA: RISK FACTORS, CLINICAL AND GEOGRAPHICAL CHARACTERIZATION, AND GENOMIC TYPING OF *COCCIDIOIDES* SPP. IN AN EMERGING ENDEMIC AREA IN NORTHEASTERN BRAZIL

Kelsen Dantas Eulálio[1], Daniel Kolath[2], Liline Maria Soares Martins[1], Antonio de Deus Filho[1], Maria do Amparo Salmito Cavalcanti[1], Lucas Machado Moreira[3], Lucas Gomes de Brito Alves[4], Daniele Yamauchi[5], Gil Benard[6], George Thompson[7], Mathieu Nacher[8], Eduardo Bagagli[5], Maria Sueli Soares Felipe[9], Bridget Barker[10], Luciana Trilles[3], Marcus de Melo Teixeira[4]

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INTRODUCTION: Coccidioidomycosis (CM) is an invasive mycosis included in WHO's list of priorities. It is endemic and notifiable in the southwestern USA but neglected in semi-arid areas in Central and South America. Interestingly, 100 years after the first report of the disease in Argentina, fewer than 1,000 total cases have been reported in South America.

METHODS: We used an unbiased and multi-institutional approach to assess whether genetic variation in the pathogen and/or in the environment affects the epidemiology of coccidioidomycosis and disease outcomes throughout the American continent.

RESULTS: We identified 292 patients with proven coccidioidomycosis between 1978 and 2021 in the Piauí and Maranhão states of Brazil, which is the largest cases series study of this disease reported so far outside the US/Mexico epidemic range. The male-to-female ratio was 57.4:1 ($p < 0.0001$). As a reflection of the predominant African American ethnicity of the Northeastern region of Brazil, most patients were also of this ancestry (78.4%) compared with Caucasians (20.9%, p=5.9E-23). There were two indigenous patients (0.7%), both from the Guajajara ethnic group in the state of Maranhão. The main reported risk factor was hunting armadillos (n = 266, 91,1%), which happened 4 to 30 days before coccidioidomycosis-related clinical symptoms. Coccidioidomycosis occurred as single cases in 63.4% (n = 185) of the instances, and as micro-epidemics, involving two to six patients (average: 2.5), in 107 instances (39.6%). Most patients (n = 271, 92.8%) presented typical acute pulmonary disease. Additionally, 10 cases (3.4%) with an initial acute pulmonary disease evolved to disseminated disease form. Seven cases (2.4%) presented the chronic pulmonary disease form and 4 cases (1.4%) the regressive pulmonary form. The most frequent clinical symptoms were cough (93%), fever (90%) and chest pain (77%). Mortality was observed in 8% of the patients that developed either severe pulmonary involvement leading to respiratory insufficiency or involvement of SNC or both. We have observed an uneven distribution of coccidioidomycosis cases over the years. In 2004 and between 2015 and 2017 we observed a spike in the cases of coccidioidomycosis in Brazil, particularly in the state of Piauí. For this state, the soil is acidic, and precipitation (p=0.015) and precipitation one-year prior (p=0.001) are significant predictors of coccidioidomycosis cases. Evolutionary analysis suggests that Brazilian strains are genotypically divergent from any *C. posadasii* reported and are nested within the TX/MX/SA clade.

CONCLUSION: We conclude that coccidioidomycosis in Northeastern Brazil has a specific infection profile, as armadillo hunters are the vast majority population at risk for acute disease. We also observe that specific bioclimatic variables such as low pluviosity and extensive droughts seem key to increasing the number of cases in Brazil. We also show that a unique *C. posadasii* genotype is circulating in Brazil, and we speculate that it might display different virulence and pathogenesis traits than other *Coccidioides* genotypes.

13 ORAL

VOLATILE MOLECULES IN HUMAN BRONCHOALVEOLAR LAVAGE FLUID CAN DISCRIMINATE VALLEY FEVER FROM OTHER LUNG INFECTIONS

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Abstract

INTRODUCTION: It is estimated there are 350,000 new cases of Valley fever each year and in endemic and highly populated regions up to 30% of community acquired pneumonia may be caused by Valley fever. It is currently difficult to diagnose, in part because symptomatic primary pulmonary infection often resembles bacterial pneumonia leading to a misdiagnosis and inappropriate treatment with antibiotics. Therefore, the primary concern in diagnosing Valley fever is distinguishing it from bacterial pneumonia, and we hypothesized that there is a suite of volatile biomarkers that can distinguish coccidioidal pneumonia from bacterial pneumonia. To test this hypothesis, we performed untargeted volatile metabolomics analysis on bronchoalveolar lavage fluid (BALF) from patients with suspected pneumonia and used machine learning to identify VOCs that discriminate between patients infected with either *Coccidioides*, another microbial lung pathogen, or no detectable infection.

METHODS: Fifty-five BALF samples from the Biospecimens Accessioning and Processing lab biorepository were provided by Tom Grys at Mayo Clinic, Phoenix, Arizona. The study was approved by the Institutional Review Boards at Arizona State University and Mayo Clinic (IRB 18-005235). Using chart review, patient samples were categorized by the infecting microbial organism(s) using the following categories: *Coccidioides*, fungal, bacterial, viral, multiple (e.g., fungal +bacterial co-infection), and negative. The human BALF samples were split into technical triplicate for VOC analyses by headspace solid-phase microextraction and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (SPME-GC×GC-TOFMS). We used Random Forest to build several models to determine if VOCs in BALF can be used to classify specimens based on the presence vs. absence of infection and the infection etiology.

RESULTS: We detected 244 VOCs in the BALF samples, eight of which could distinguish *Coccidioides* pneumonia from non-Cocci infected samples, and specifically from bacterial pneumonia. Our pilot data suggest that the BALF of patients with primary pulmonary Valley fever can be distinguished from the BALF of patients with other forms of CAP using their volatile profiles.

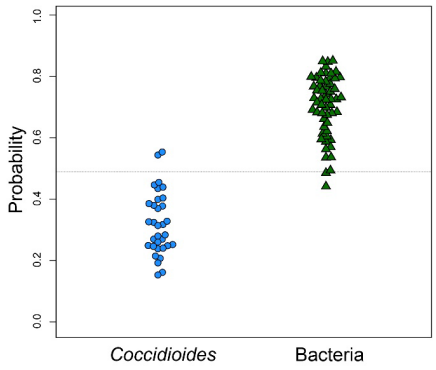

Figure 1. Random Forest classifies *Coccidioides*-positive vs. bacteria-positive samples using seven human BALF volatile metabolites with a model accuracy of 0.95. Bee swarm plot depicting class probabilities (*Coccidioides* versus Bacteria); each point represents a technical replicate of a BALF sample, grouped into their true classifications of *Coccidioides* (blue circles) and Bacteria (green triangles), and plotted based on the probability that the sample is classified as Bacteria

based on 100 iterations of Random Forest. Points towards the top of the plot indicate a higher probability of classifying as Bacteria, while those towards the bottom of the plot indicate a higher probability of classifying as *Coccidioides*.

CONCLUSIONS: Our results suggest that a VOC breath test for Valley fever is a viable option, the next steps of this work will be to collect the breath from persons with community-acquired pneumonia and determine which of the Valley fever biomarkers can differentiate between bacterial and fungal etiologies of disease.

14 ORAL

POSITIVE ASSOCIATION BETWEEN FINE MINERAL DUST EXPOSURE AND COCCIDIOIDOMYCOSIS INCIDENCE IS MODIFIED BY ANTECEDENT CLIMATE CONDITIONS

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Abstract

Introduction: Coccidioidomycosis, caused by inhalation of *Coccidioides* spores, is an emerging infectious disease that is increasing in incidence throughout the southwestern United States. The pathogen is soil-dwelling, and spore dispersal and human exposure are thought to co-occur with airborne mineral dust exposures, yet fundamental exposure-response relationships have not been conclusively estimated. We estimated associations between fine mineral dust concentration and coccidioidomycosis incidence in California from 2000-2017 at the census tract-level, estimated heterogeneity in exposure-response across seasons and regions, and examined potential modification by antecedent climate conditions.

Methods: We acquired monthly census tract-level incidence data and speciated fine mineral dust concentrations from 2000 to 2017. We fitted zero-inflated distributed lag non-linear models to estimate the overall exposure-lag-response relationships and identified factors contributing to heterogeneity in exposure-response. Using a fixed-effects meta-analysis approach, we estimated county-specific exposure-responses for cumulative exposures to fine mineral dust and pooled effects.

Results: We found a positive exposure-response relationship between cumulative mineral dust exposure in the one to three months before disease onset and coccidioidomycosis incidence across the study region (IRR for a 1 $\mu g/m^3$ increase relative to 0.1 $\mu g/m^3$ = 1.96 [95% CI: 1.78, 2.15]). Consistent, positive associations were observed between coccidioidomycosis incidence and fine mineral dust exposures one (IRR for a 1 $\mu g/m^3$ increase relative to 0.1 $\mu g/m^3$ = 1.19 [95% CI: 1.15, 1.23]), two (IRR = 1.21 [95% CI: 1.15, 1.27]), and three (IRR = 1.11 [95% CI: 1.07, 1.16]) months before disease onset. The cumulative exposure-response relationship varied significantly by county (lowest IRR: 0.85 [95% CI: 0.56, 1.30]; highest IRR: 2.69 [95% CI: 1.98, 3.66]), with mineral dust exposures within highly endemic counties posing the greatest risk. Prior climate conditions were modest effect modifiers.

Conclusions: Lagged exposures to fine mineral dust were strongly associated with coccidioidomycosis incidence in the endemic regions of California from 2000-2017.

15 ORAL

X-LINKED LYMPHOPROLIFERATIVE SYNDROME TYPE 1 AND CTPS1 DEFICIENCY – TWO INBORN ERRORS OF IMMUNITY PRESENTING WITH SERONEGATIVE COCCIDIOIDOMYCOSIS

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Abstract

Introduction: Monogenic disorders impairing the interferon gamma (IFN-γ) and interleukin 12 (IL-12) signaling axis have classically been associated with increased susceptibility to disseminated coccidioidomycosis. We report the first known cases of XLP-1 and CTPS1 deficiency presenting with seronegative coccidioidomycosis.

Methods: We performed a case series of 2 patients after obtaining local IRB exemption.

Results: *Patient 1* is a 5-year-old male with moderate atopic dermatitis, recurrent herpes simplex virus (HSV) stomatitis, severe cutaneous molluscum, chronic diarrhea, and recurrent sinopulmonary infections who was admitted for evaluation of a 1-month history of recurrent fever of unknown origin. His initial laboratory tests were significant for anemia; however, serum electrolytes and liver enzymes were unremarkable. Serum IgG, IgA, and IgM all were within the age-adjusted reference range. CD8+ T cells, CD19+ B cells, and natural killer cells were decreased; however, CD4+ T cells were within the reference range. He had 3 of 13 vaccine titers ≥1.3 µg/mL post–13-valent pneumococcal conjugate vaccine (PCV13) series, consistent with suboptimal seroconversion. He had low lymphocyte response to phytohemagglutinin, concanavalin A mitogens, and Candida and tetanus antigens, but normal response to pokeweed mitogen. Notably, his coccidioidal serology (enzyme-linked immunoassay) resulted negative for both IgG and IgM and complement fixation titer was also negative. Computed tomography (CT) of the chest showed a necrotizing lesion in the right upper lobe and bulky hilar, and mediastinal lymphadenopathy. Culture from interventional radiology–guided lung biopsy grew Coccidioides immitis species. He was started on intravenous fluconazole (10 mg/kg/day) and intravenous immunoglobulin 0.5g/kg every 4 weeks. Lumbar puncture and bone imaging rule out dissemination. Targeted immunodeficiency genetic testing identified a pathogenic homozygous splice acceptor variant in *CTPS1*, c.1692-1G > C, confirming CTPS1 deficiency. 7 months later the patient successfully underwent allogeneic hematopoietic stem cell transplantation for his immunodeficiency.

Patient 2 is a 13-year-old male with moderate persistent asthma, recurrent otitis media requiring four sets of tympanostomy tubes, chronic sinusitis, and common variable immunodeficiency (CVID) presented with acute-on-chronic hypoxic respiratory failure. He had been off subcutaneous immunoglobulin (SCIg) replacement for 2 years. Sputum culture was positive for H. influenzae, treated with cefepime and azithromycin and supplemental oxygen for acute hypoxic respiratory failure. Fever persisted despite 48 hours of antibiotics. CT chest showed bronchiectasis with mediastinal, subcarinal and hilar adenopathy. Coccidioides immitis IgG and IgM were negative, but urine antigen was positive. There was no radiologic or cerebrospinal fluid evidence of disseminated coccidioidomycosis. Immunologic workup revealed undetectable IgG, IgA, IgM, and IgE. Lymphocyte subsets showed severe T and NK cell lymphopenia. Total B cell count was normal, but he had decreased CD19+27+ and transitional B cells, with absent switched memory B cells, and no plasmablasts. Lymphocyte proliferation to mitogens/antigens was decreased. Serum ferritin was normal, and EBV PCR was negative. A targeted 429-gene panel identified a hemizygous variant of uncertain significance (VUS) in *SH2D1A*, c.82_102dup (p.Ser28_Ser34dup). SAP expression was absent in CD4+ T cells and NK cells with a bimodal expression in CD8+ T cells, suggesting possible mosaicism in this subset. He was diagnosed with X-linked lymphoproliferative disease 1 (XLP-1) and treated with fluconazole, trimethoprim-sulfamethoxazole prophylaxis, and IgG replacement (1 g/kg intravenous initially, then transitioned to weekly SCIg targeting an IgG >1000 mg/dL), with clinical improvement, resolution of fevers and acute hypoxia. He was referred for hematopoietic cell transplant evaluation.

Conclusion: XLP-1 is characterized by hemophagocytic lymphohistiocytosis (HLH), lymphoma, and hypogammaglobulinemia. Cytidine nucleotide triphosphate synthetase 1 (CTPS1) deficiency is characterized by early-onset severe recurrent viral infections, particularly to Epstein-Barr virus (EBV) and varicella zoster virus, as well as recurrent sinopulmonary infections with encapsulated bacteria. Genetic testing for IEIs may be warranted in younger patients who present with seronegative coccidioidomycosis.

17 POSTER

A CASE OF DISSEMINATED COCCIDIOIDOMYCOSIS TREATED WITH OLOROFIM

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Abstract

Introduction: Olorofim is a novel antifungal currently under review by FDA for the treatment of invasive fungal infections. We describe a case of disseminated coccidioidomycosis treated with olorofim after fluconazole and Itraconazole failure.

Methods: Retrospective case review with IRB approval and patient consent.

Results: A 37-year-old woman with recently diagnosed pulmonary coccidioidomycosis (cocci) was referred to Valley Fever Institute (VFI) at Kern Medical due to a lack of response and dissemination. Five months prior, she had been diagnosed with acute pulmonary cocci at an outside facility with recurrent episodes of hypoxia after the failure of symptomatic management for presumptive viral etiology. Upon diagnosis, her cocci complement fixation (CF) titer was 1:16 and she was started on 600 mg of fluconazole daily. Two months later she had severe complaints of productive cough, chest pain during deep inspiration, subjective fevers, night sweats, chills, body aches, and arthralgias of both hands and bilateral lower extremities. Fluconazole was increased to 1000 mg daily resulting in a therapeutic level at 63.5 mcg/mL; however, her body aches, and arthralgias continued to worsen resulting in significant impacts to her activities of daily living including difficulty walking, difficulty maintaining employment, and inability to spend quality time with family. When sero-reactivation of her cocci CF titer to 1:128 raised concern for dissemination, treatment was changed to itraconazole 200 mg three times daily. Over the next 4 months, her symptoms did not improve and she was referred to VFI. A whole-body bone scan showed increased foci in left ninth and right eighth ribs representing osseous dissemination.

Given the failure of therapy with fluconazole and itraconazole and limited therapeutic options due to insurance coverage, she was initiated on treatment with olorofim at 90 mg twice daily through a clinical trial (NCT03583164). By Week 7 of olorofim, she was symptomatically improved and her cocci CF titer had fallen to 1:16. During evaluation of an acute chest syndrome that developed during Week 10, resolved during Week 11, and was retrospectively attributed to *H. influenzae*, the olorofim dose was increased to 120 mg twice daily because of visualization of misshapen spherules in BAL fluid. While on olorofim monotherapy, improvements were noted in the trend of her cocci CF titers down to CF of 1:4, with complete resolution of her previously debilitating symptoms. As of February 2023, she has been receiving olorofim for 14 months and is able to exercise, cook, and spend quality time with family.

Conclusion: Olorofim monotherapy resulted in significant improvement in serology, clinical symptoms, and quality of life in a patient with disseminated coccidioidomycosis who had failed 8 months of treatment with fluconazole and itraconazole. Clinicians should be aware of novel antifungals in the treatment of this debilitating disease.

18 ORAL

OLOROFIM FOR TREATMENT OF INVASIVE FUNGAL INFECTIONS (IFI) DUE TO COCCIDIOIDOMYCOSIS IN PATIENTS WITH LIMITED OR NO TREATMENT OPTIONS: INTERIM RESULTS FROM A PHASE 2B OPEN-LABEL STUDY (NCT03583164, STUDY 32)

GR Thompson[1], Martin Hoenigl[2], Jeff Jenks[3], Sanjay Mehta[4], Bennett Penn[1], Stuart Cohen[1], Mark Bresnik[5], John Rex[5]
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Abstract

Introduction: Olorofim, the first orotomide antifungal, selectively inhibits fungal dihydroorotate dehydrogenase (DHODH), a key enzyme in fungal pyrimidine biosynthesis. Olorofim is active against Aspergillus (including azole-resistant and cryptic species), resistant molds (e.g., Lomentospora prolificans, Scopulariopsis) and dimorphic fungi (e.g., Coccidioides). The drug is given orally and cleared by multiple CYP450 isoenzymes.

Methods: Patients with refractory extrapulmonary coccidioidomycosis were included in an open-label, single-arm, 12-week Phase 2b study of olorofim for invasive fungal infections in patients with limited or no treatment options (NCT03583164). Olorofim was given po as a loading dose of 150mg BID on Day 1, followed by 90mg BID with extended therapy beyond Week 12 allowed. The primary endpoint was Data Review Committee (DRC)-adjudicated overall response rate by pathogen at Day 42 using the EORTC-MSG response criteria. Efficacy outcomes were assessed at multiple timepoints by the DRC and Investigator, including response determined by Investigator at Day120. The clinical response outcomes for the first 11 DRC-adjudicated coccidioidomycosis patients are presented.

Results: There were 11 patients with coccidioidomycosis included at database lock for the first 100 study cases. Nine male, 2 female; median age 45 yrs (range 28-66); Median duration of disease from diagnosis to study entry was 702.0 days (range: 201-3231). CNS dissemination was present in 10/11. Other concurrent sites of disease included 1 peritoneal, 1 bone (spine), 2 joint, 1 lung, and 2 skin/soft tissue.

Clinical Response per EORTC-MSG criteria

Adjudication (N=11)	Day 42 (DRC)	Day 84 (DRC)	Day 120 (Investigator)
Complete	2 (18.2%)	1 (9.1%) *	4 (36.4%)
Partial	5 (45.5%)	6 (54.5%)	3 (27.3%)
Total (CR + PR)	7 (63.6%)	7 (63.6%)	7 (63.6%)

* Of the two patients with Complete Clinical Response at Day 42, one had a missing visit at Day 84 and is thus counted as a failure. At Day 91 (1 day outside the Day 84 Visit window) and beyond to Day 722 (database lock), the patient has remained completely asymptomatic.

The timeframe in which clinical responses were achieved was rapid (approximately 1.5 to 4 months) and unexpected, given the duration of cocci disease and the persistence of significant, debilitating symptoms despite all available treatment options at study entry.

Olorofim was well tolerated in the overall study population (N=100) over the 84d median dosing duration (max 722 days). Altered hepatic biochemistry possibly due to olorofim was seen in 8% of these patients and managed by dose reduction/pause and led to discontinuation in 2%. Mild, self-limiting GI intolerance was noted in 2%.

Conclusion: Olorofim is an oral, novel mechanism antifungal agent with activity against a range of mold and dimorphic fungal infections which are difficult to treat. Initiation of olorofim led to complete or partial clinical response in close to two-thirds of patients with limited or no treatment options for refractory extrapulmonary coccidioidomycosis. Olorofim has a positive benefit-risk profile in this limited and well-defined population of patients.

19 ORAL

TOWARD BETTER TREATMENTS: INVESTIGATING FUNGAL SECRETED PROTEASES IN COCCIDIOIDOMYCOSIS

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Abstract

INTRODUCTION: Coccidioides, a dimorphic fungal pathogen endemic to the Southwest United States, Central, and South America, infects and kills immunocompetent individuals. Limited treatment options render coccidioidomycosis, the infection caused by Coccidioides, a cause of unacceptably high morbidity and mortality. We urgently need better treatments. Coccidioides' virulence stems from its unique and poorly understood host form, the spherule. Arthroconidia are inhaled by the host and develop into spherules. Once mature, a spherule is filled with hundreds of endospores. Spherules then rupture and disseminate endospores within the host. As spherules are unique to Coccidioides, most spherule biology remains unknown. Interestingly, the Coccidioides genomes encode two expanded families of proteases, the subtilases and the deuterolysins, that are promising treatment targets for coccidioidomycosis. Protease inhibitors have been successfully developed as treatment for multiple diseases, from HIV to cancer, but their promise has not yet been leveraged for fungal infections.

METHODS: To demonstrate the biologic significance of these protease families, we have studied spherulation in the presence of AEBSF (a serine protease inhibitor) and 1,10-phenanthroline (a metalloprotease inhibitor) and used microscopy to characterize the developmental consequences of protease inhibition. We have examined deuterolysin and subtilase expression over the entire spherulation cycle in triplicate samples, including timepoints with endospore release in Converse media, at 39˚C, 10% CO_2. Additionally, we have examined deuterolysin and subtilase expression in mycelial growth in Converse media.

RESULTS: For the first time, we have inhibited spherule formation by treating Coccidioides with a protease inhibitor that blocks subtilase activity, indicating the importance of the subtilases in the parasitic phase of Coccidioides biology. We have found that subtilase and deuterolysin expression is induced upon spherule formation, in four patterns: 1) during early formation of spherules, 2) continuous increase over the spherulation cycle, 3) two distinct peaks of expression in arthroconidia and endospore stages, or 4) dramatic increase in expression at the time of endospore release. One gene, Sub1, fits the fourth pattern, and is in fact one of the top 10 most abundant transcripts in the spherule at the time of endospore release. Phenotypic characterization of this mutant is underway and molecular work is ongoing to determine the substrates of these two protease families.

CONCLUSION: By linking protease activity to spherulation, we have demonstrated the importance of further dissecting the role of these two expanded protease families in Coccidioides virulence. Furthermore, these results support inhibition of the subtilase family as a possible therapeutic strategy for coccidioidomycosis.

20 POSTER

NON-SURGICAL MANAGEMENT OF DISSEMINATED COCCIDIOMYCOSIS BASILAR MENINGITIS WITH COMMUNICATING HYDROCEPHALUS

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Abstract

INTRODUCTION: Disseminated coccidioidomycosis involving the meninges is fatal if untreated, typically requiring lifelong antifungal therapy and nearly inevitable neurosurgical intervention. The standard of care for coccidioidomycosis meningitis with symptomatic hydrocephalus involves ventriculoperitoneal (VP) shunt placement for definitive diversion of cerebral spinal fluid.

We present a case of an immunocompetent young male who declined invasive treatment and opted exclusively for medical management of his newly diagnosed coccidioidomycosis meningitis with communicating hydrocephalus. This controversial approach to management differs from the Infectious Disease Society of America (IDSA) recommendations. However, our patient's clinical and radiographic improvement on oral fluconazole alone indicates that non-surgical management may be an option for minimally symptomatic, highly functional patients. Additionally, we discuss the associated implications for outpatient general practitioners who must closely monitor these patients electing to forgo invasive shunt surgery.

CASE PRESENTATION: A previously healthy 29-year-old Canadian male of Indian descent presented to our emergency department (ED) with a six-month history of persistent headaches, a 20-pound unintentional weight loss, and bilateral papilledema noted on a routine optometry exam. He initially developed severe headaches a few weeks after briefly traveling to Arizona for a materials engineer job interview in the spring of that year. Upon returning to Canada, he sought medical attention for new-onset, debilitating headaches at two Canadian EDs, but his workup and neuroimaging were unremarkable. His symptoms were attributed to migraines, for which he was offered abortive therapy before discharging home. The patient never sought further care from outpatient providers for his headaches before relocating to Arizona for his new job.

In our ED, computed tomography (CT) of the head showed ventriculomegaly. Subsequent magnetic resonance imaging (MRI) of the brain confirmed extensive basilar meningeal enhancement with communicating hydrocephalus. CT thorax identified a 3 mm left lower lobe pulmonary nodule. Cerebrospinal fluid (CSF) studies showed low glucose (19 mg/dL), elevated protein (279.8 mg/dL) with lymphocytic predominant leukocytosis (87% of 335 per µL), and an elevated opening pressure of 33 cmH2O. Serum Coccidioides immunodiffusion (IMDF) IgM and IgG were detected and Coccidioides complement fixation (CF) titer was 1:32 in the CSF and 1:128 in the serum, confirming the diagnosis of coccidioidomycosis meningitis. Treatment with intravenous amphotericin B for five days with concurrent intravenous fluconazole was initiated until therapeutic azole levels reached blood-brain barrier penetrance concentrations.

Our patient was offered definitive treatment for increased intracranial pressure (ICP) with VP shunt placement according to current IDSA guidelines. However, he declined invasive procedures due to his perception that his symptoms were not hindering his ability to perform his highly demanding occupational and daily activities. Therefore, he was transitioned to oral fluconazole before safely discharging home with close outpatient follow-up in subspecialty clinics while awaiting an initial establishing care visit with a primary care provider (PCP). Since discharge, he endorses a tolerance of oral fluconazole with symptomatic and radiographic improvement on medical therapy alone.

CONCLUSION: This case illustrates a controversial approach to outpatient management of basilar coccidioidomycosis meningitis with communicating hydrocephalus and increased ICP in an immunocompetent host. For patients capable of regular outpatient follow-up and routine clinical monitoring with regular laboratory, medical studies, and neuroimaging, delaying VP shunt placement may be a viable option. In this multidisciplinary outpatient approach to caring for patients with coccidioidomycosis meningitis, PCPs play an integral role in mitigating fatal complications of this lifelong infection that may eventually require urgent neurosurgical intervention when the disease progresses and clinical decompensation occurs.

21 ORAL

FAILURE TO DETECT ΔCPS1 IN A MOUSE REVERSION-TO-VIRULENCE STUDY

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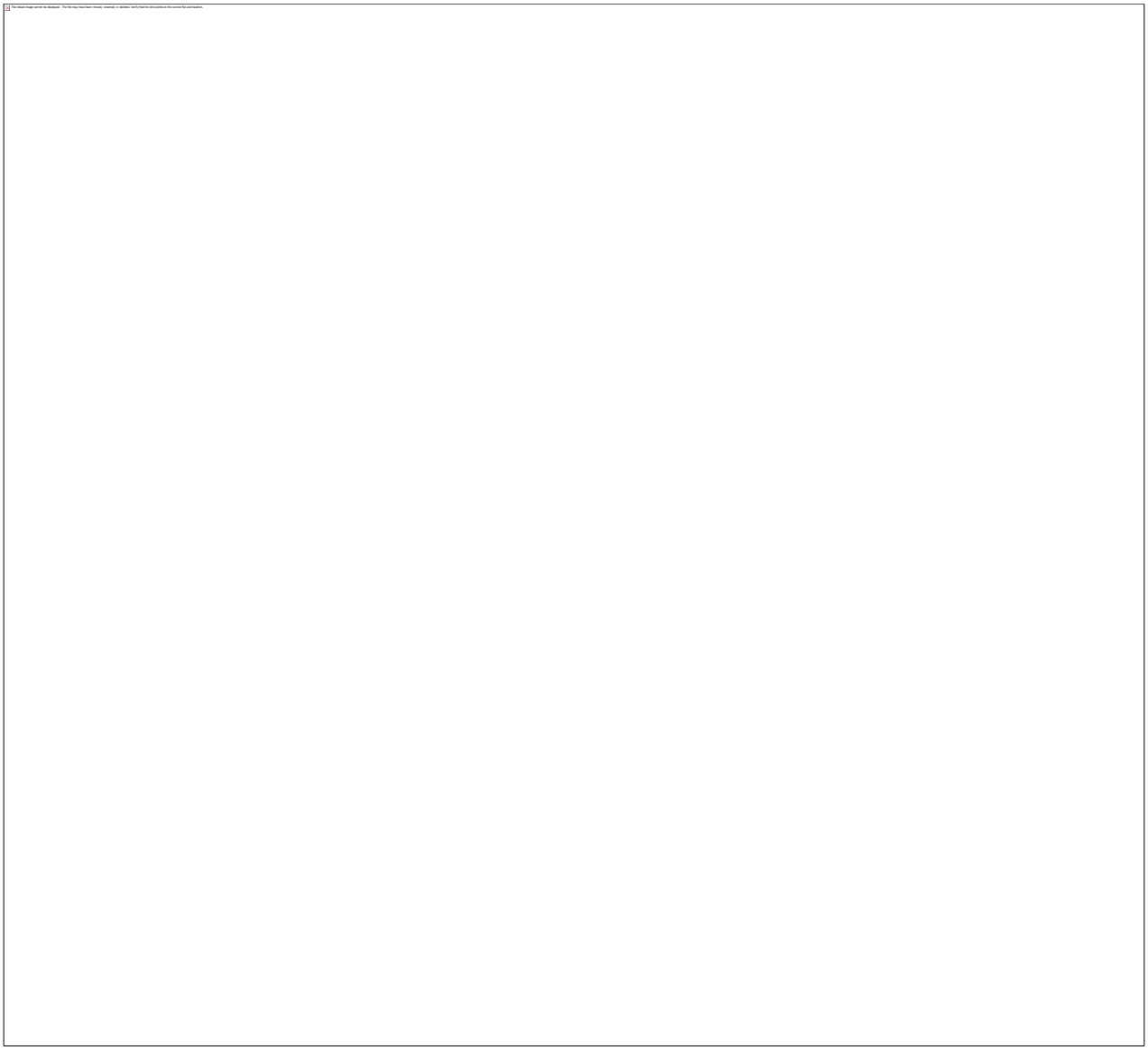
Abstract

INTRODUCTION: Safety studies for licensing of veterinary vaccines include backpassage/reversion to virulence studies. This measures recovery of the vaccine organisms from animals inoculated with Master Seed by the most susceptible route above the minimum immunizing dose, followed by serial passage into subsequent groups of animals. This design seeks to determine whether the vaccine strain regains function and pathogenicity. We piloted this in a mouse model by the most susceptible infection route according to APHIS Veterinary Services Memorandum 800.201 as a precursor to conducting a study in dogs.

METHODS: On study day 0, C57BL/6nj (8 wk F) mice were inoculated intranasally (IN) with 10,000 or 30,000 Δcps1 spores (N=20 mice each). On days 7, 14, 21, and 28, 5 mice from each group were sacrificed and lungs homogenized individually in 1 mL of saline. 1:10 and 1:100 serial dilutions plus 400 uL of the undiluted homogenate were cultured on GYE agar plates at 37ºC for colony quantitation. On days 7, 14, and 28, 500 uL of undiluted homogenate from each mouse was pooled by group and filtered through a 200 µm cell strainer, washed once in water with centrifugation, and then resuspended in 400 µL of sterile, isotonic saline. Ten mice each were inoculated intranasally with washed homogenate from the 10,000 spore and 30,000 spore groups. Five mice each were sacrificed at 14 days and 28 days following backpassage inoculation and the entire lung homogenate cultured to enumerate colonies. [Figure 1] [IACUC #14-562]

RESULTS: From the initial inoculations, we recovered Δcps1 colony-forming units (cfu) from 3 of 20 mice inoculated with 10,000 spores (range 0-29 cfu/mouse; median 0 cfu) and 4 of 19 mice given 30,000 spores (range 0-79 cfu/mouse; median 0 cfu). [Table 1] Mean pooled homogenate concentrations were ≤50 cfu/mL (range 0-50) for IN instillation into backpassage mice. No Δcps1 was recovered from lungs of mice sacrificed 14 and 28 days after inoculation with pooled homogenates of first passage mice. Mice had no clinical signs of coccidioidomycosis.

CONCLUSIONS: We have previously reported that Δcps1 replicates poorly in vivo and is avirulent, making it a good candidate for a live vaccine. Mice inoculated IN with Δcps1 doses in the range that provide strong vaccine protection showed no reversion to virulence. On backpassage performed according the USDA APHIS guidelines, Δcps1 was not recovered from mice inoculated with lungs of Δcps1-infected mice, verifying its avirulence in mice.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22 ORAL

INTESTINAL AND TRACHEAL MICROBIOMES INHIBIT IN VITRO GROWTH OF *COCCIDIOIDES IMMITIS*

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Abstract

INTRODUCTION: Valley fever is a disease caused by Coccidioides, a fungal pathogen, and is frequently misdiagnosed as community acquired bacterial pneumonia and treated with several rounds of antibiotics prior to accurate diagnosis. Antibiotic treatment changes the microbiome repertoire and host immunity; the impact of these microenvironmental changes on Coccidioides invasiveness is unknown. A soil microbial antagonist related to Bacillus subtilis inhibits in vitro growth of Coccidioides immitis through a clear zone of inhibition between fungi and bacteria (Lauer et. al 2019). Whether host microbiota can also inhibit Coccidioides is unknown.

METHODS: To assess whether the host microbiota has inhibition capabilities against Coccidioides, we performed two types of inhibition assays. A 50/50 inhibition assay was performed in which the host microbiota and Coccidioides were placed in direct competition simultaneously on agar plates. A spike in inhibition assay was also performed in which the host microbiota was allowed to reach ~80% confluency, to mimic an established in vivo microbiome, before spiking in Coccidioides. To assess if partial clearance of the host microbiota would allow a niche for Coccidioides growth, a 4-antibiotic cocktail was used to perform disc diffusion spike in assays. The area of growth was observed and measured at day 4, 7, and 11 for all inhibition assays. 16S rRNA sequencing of tracheal and intestinal growth on the three media types was performed to identify differences in selective bacterial growth.

RESULTS: Our in vitro data indicate that intestinal and tracheal host microbiome species grown on 2xGYE and CNA with 5% sheep's blood agar inhibit Coccidioides growth, but not intestinal microbiota grown on chocolate agar. Partial in vitro depletion by antibiotic disc diffusion assays revealed that partial depletion of host microbiota allows greater Coccidioides growth compared to an undisturbed microbiota. 16S rRNA sequencing revealed genus level differences in growth between media.

CONCLUSION: Our data suggests that depleting commensal microbiomes, allows a niche for Coccidioides growth. These in vitro findings could have clinical relevance and shape the way physicians assess prescription of antibiotics and coccidioidomycosis diagnosis. In vivo antibiotic treatment and infection experiments are ongoing.

24 ORAL

Opportunities for Valley fever researchers and demystifying the NIH Grants process

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Abstract

INTRODUCTION: The National Institute of Allergy and Infectious Diseases (NIAID) funds one of the largest medical mycology research portfolios covering all invasive human fungal pathogens, including *Coccidioides* spp. Valley fever is a priority area of research for NIAID.

METHODS: A Notice of Special Interest (NOSI) was recently published to highlight NIAID's interest in supporting research in the areas outlined in the NIAID Strategic Plan For Research To Develop A Valley Fever Vaccine. NIAID has a suite of preclinical services supporting therapeutic, diagnostic and vaccine development. These services are free and available to investigators in academia, not-for-profit organizations, industry, or governments worldwide. For research areas outside of vaccine development, researchers can utilize the investigator-initiated granting mechanisms. Additionally, there is a NOSI supporting an NIH-wide Climate Change and Health Initiative (CCHI) that may be applicable to understanding Coccidioides geographic range and changes in disease incidence.

RESULTS: The Coccidioidomycosis portfolio has grown in recent years as researchers have taken advantage of funding opportunities for Valley fever research.

CONCLUSION: NIH granting mechanisms can be complicated. Tips and tricks for navigating the NIAID/NIH application process and preclinical services will be discussed.

25 POSTER

MILIARY REACTIVATION OF COCCIDIOIDOMYCOSIS: 5 YEARS IN THE MAKING

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Abstract

INTRODUCTION: Coccidioidomycosis is a fungal infection that primarily affects the pulmonary system. Only a small percentage, approximately 1%, of pulmonary coccidioidomycosis disseminates. Reactivation of coccidioidomycosis after the initial infection is also uncommon and when it does occur is usually seen in immunocompromised patients. Here we describe a 54-year-old man with uncontrolled diabetes who experienced a reactivation of coccidioidomycosis 5 years after initial infection with miliary, osseous, synovial, and soft tissue dissemination.

METHODS: Ethical approval for this single patient case report was obtained from the Kern Medical Institutional Review Board (IRB #22105) and a retrospective chart review was conducted.

RESULTS: The patient is a 54-year-old Latino man with poorly controlled diabetes who is a field worker from the central valley of California. He had been previously diagnosed with bilateral diffuse pulmonary coccidioidomycosis at our institution years prior. The diagnosis was made with positive sputum culture for *Coccidioides immitis* and positive IgM and IgG immunodiffusion serology with complement fixation (CF) titer of 1:4. He received 4 weeks of Liposomal amphotericin B (LAmB) due to the extension of his illness and hypoxemia. Treatment was changed to oral fluconazole and he was followed for one year with complete resolution of chest radiographic findings however was unfortunately lost to follow up thereafter.

Five years later he again presented to our institution with cough, generalized weakness, fever, and right knee pain and swelling. Chest imaging revealed a classic miliary pattern and coccidioidomycosis CF titer significantly increased to 1:64. Right knee MRI with contrast demonstrated abnormal distal femur and proximal tibia marrow enhancement consistent with osteomyelitis as well as rim-enhancing intraosseous fluid collections, large joint effusion, and 5 x 3 cm and 14 x 6 cm complex multiloculated peripherally enhancing fluid collections consistent with abscesses in the lateral vastus and gastrocnemius muscles respectively. Arthrocentesis of the knee and aspiration of the fluid pockets were collected for fungal cultures. Clinical diagnosis of reactivation coccidioidomycosis with osseous, synovial, and soft tissue dissemination plus miliary pattern was made. Patient was subsequently placed on LAmB regimen with plan to continue for 12 weeks and consultation with orthopedics was obtained for incision and drainage of the knee and posterior lower extremity fluid collections.

CONCLUSION: Although rare, coccidioidomycosis reactivation can occur. Reactivation typically presents as miliary disease or extrapulmonary dissemination. Risk factors for reactivation include immunosuppression, such as uncontrolled diabetes, and incomplete treatment courses. It is critical for clinicians to continue serial monitoring of clinical symptoms and CF titers after initial therapy is discontinued, especially in those with immunocompromising conditions. A prolonged course of treatment and even lifetime therapy might be necessary.

26 POSTER

USE OF A WEB-BASED SURVEY TO ASSESS TREATMENT OUTCOMES IN DOGS WITH COCCIDIOIDOMYCOSIS

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Abstract

INTRODUCTION: There is scant information in the literature about the outcomes of dogs being treated for coccidioidomycosis. The Valley Fever WOOF (Web-based Observations, Outcomes, and Findings) Study is currently running to collect owner reported data on the response to azole therapy the first year after diagnosis.

METHODS: A series of online surveys were designed using REDCap (Research Electronic Data Capture). A public survey collects information about signalment, clinical signs, diagnostics, and medications. In order to be enrolled, dogs must have received a coccidioidomycosis diagnosis within the previous 30 days and be treated with an oral azole. Dogs were denied enrollment if the dog was experiencing recurrent disease or if no diagnostics were performed. Owners of enrolled dogs receive individual links to follow up surveys about response to therapy and changes in medications at 1, 2, 3, 6, 9, and 12 months post-enrollment. Owners are also questioned about changes in the dog's status, such as rehoming or death, and whether this was associated with coccidioidomycosis.

RESULTS: The WOOF study opened in March 2022. There are currently 57 dogs enrolled, with most (91.2%) dogs in AZ. The remaining dogs reside in CA, NV, TX, or WA. Enrolled dogs ranged in age between 0.4 and 14 years of age (mean = 5.3 years). The average weight of dogs at diagnosis was 22.5 kg (range 3.2-57.7 kg). At diagnosis, AGID titers ranged from negative to 1:128 (median = 1:16). Twenty-seven percent of dogs with follow up titers performed had a decrease of ≥ 2 dilutions by 9 months post-diagnosis. The most common clinical signs were lethargy (75%), cough (64.3%), and poor appetite (51.8%). Lethargy and poor appetite were reported to be completely resolved in 59% of the dogs with follow up data; almost all reported resolution within 3 months of diagnosis. Forty-five percent of dogs that had cough as an initial clinical sign had complete resolution of coughing by three months. Dogs were initially treated with either fluconazole (91.2%, average dose 9 mg/kg q12h) or itraconazole (8.8%, average dose 8.2 mg/kg/day). A change in antifungal drug was reported for five dogs: 2 for adverse effects and 3 for failure to respond. Four (7%) dogs were reported deceased, with all owners stating that the dogs were euthanized due to progression of Valley Fever.

CONCLUSIONS: The WOOF study aims to prospectively evaluate owner-reported outcomes for dogs with coccidioidomycosis. The goal is to provide veterinarians with data about the length of time to improvement of clinical signs, the value of serial monitoring of immunodiffusion titers, and tolerance of azole antifungal drugs.

27 POSTER

WHAT YOU SEE IS NOT WHAT YOU GET: GERM CELL TUMOR MIMICKING DISSEMINATED COCCIDIOIDOMYCOSIS

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Abstract: We present the case of a 30-year-old male with history of recently diagnosed disseminated coccidioidomycosis who presented to the hospital with a myriad of symptoms including shortness of breath, generalized weakness, lower limb weakness and urinary retention. He was recently diagnosed with “disseminated coccidioidomycosis” by an outside provider on an outpatient basis and started on fluconazole orally. However, due to a lack of improvement and significant symptoms, he was sent to the hospital to initiate liposomal amphotericin B treatment. After a comprehensive workup, an alternative diagnosis was suspected and eventually confirmed as metastatic germ cell carcinoma. Due to the vast dissemination and his poor functional status despite chemotherapy initiation, the patient elected for palliative care and expired shortly after at hospice. This case demonstrates the similarity of clinical findings between disseminated infections and malignancies.

Introduction: Coccidioidomycosis is a common infection in southwestern United States. It causes a wide range of presentations from asymptomatic to disseminated coccidioidomycosis (DC). On imaging, DC presents as multiple nodular opacities. It is important to consider other diseases with similar symptoms and appearance on imaging in the differential diagnosis of DC.

Case presentation: A 30-year-old Hispanic male with history of disseminated coccidioidomycosis presented to the hospital complaining of a two-week history of shortness of breath, chest pain on deep inspiration, generalized weakness and dry cough. Patient also endorsed lower back pain with bilateral lower extremity weakness, and bowel and urinary retention. He was being managed outpatient with antifungals for disseminated coccidioidomycosis.

Upon presentation, vital signs were significant for tachycardia (heart rate of 128) and tachypnea (respiratory rate of 24). Physical examination revealed decreased breath sounds on the right side, decreased sensation in both legs and groin and weakness in lower extremities. A right neck enlarged lymph node was also noted. Chest X-ray revealed moderate right pleural effusion and left upper zone nodular opacities. CT and MRI showed numerous bilateral solid nodules, moderate right pleural effusion and widespread sclerotic and sclerotic/lytic lesions throughout the skeleton, some compressing the lumbar spine.

Antifungals were started but coccidioidomycosis serology showed IgM non-reactive and an alternative diagnosis of malignancy was considered. A biopsy of the anterior neck lymph node showed germ cell neoplasm (GCN) and thoracentesis showed exudative fluid. Ultrasound of the testes showed no abnormalities and the patient was started on chemotherapy for extragonadal GCN.

Patient later elected for palliative care and passed away shortly after.

Discussion: Disseminated coccidioidomycosis may involve skin, joints, bones or CNS. Other differential diagnoses manifesting as spread out nodules should also be considered. DC and GCN with bone metastases can be difficult to differentiate. Both present with bone pain, mass effect, and weight loss. Clues that favor one diagnosis over the other should be investigated.

Testicular involvement in DC is uncommon and should raise suspicion of GCN. Travel history to central California would favor DC and coccidioidomycosis serology can also be a strong diagnostic tool. Biopsy of the lesion remains the definitive diagnosis if malignancy is suspected.

Conclusion: Disseminated coccidioidomycosis and germ cell carcinoma share a very similar presentation. High suspicion of one should raise suspicion of the other.

28 ORAL

CREATION AND EVALUATION OF AN MRNA VACCINE AGAINST COCCIDIOIDOMYCOSIS

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[2] Program in Molecular Medicine. The UMass Chan Medical School

Introduction: Development of a mRNA vaccine against *Coccidioides* infection is an attractive vaccination strategy in large part due to the speed and low cost of manufacturing. Previous work in our lab using transgenic mice expressing the human MHC class II allele DRB1*0401 has shown that immunization with the multivalent antigen rCpa1 loaded in glucan-chitin particles (GCPs) as a delivery system and adjuvant is protective for mice intranasally challenged with a potentially lethal doses of *Coccidioides* spores. In this study, we will create, optimize, and evaluate an mRNA vaccine employing a construct encoding a *Coccidioides*-specific antigen we refer to as antigen X (AgX) as an effective vaccination strategy. The mRNA vaccine will be delivered using the GCPs that can stimulate a protective Th1 and Th17 immunity but have not been shown to successfully delivered mRNA. Here we confirm that GCP is an effective and appropriate delivery system for an mRNA vaccine against *Coccidioides* infection.

Materials and Methods: An mRNA construct comprised of the sequence encoding the *Coccidioides*-specific AgX was codon-optimized for expression in mammalian cells and the synthesis was subsequently carried out by TriLink. The resulting mRNA, designated AgX, was encapsulated into GCPs containing 0 ug, 1 ug, 5 ug, or 10 ug of the construct. First, we vaccinated mice with a dose of 5 ug of mRNA by the subcutaneous route and single cells were prepared from the injection sites, draining lymph nodes, spleens, and bone marrow at 36 hr post vaccination for determining *in vivo* expression of AgX in phagocytes by flow cytometry analysis. Furthermore, C57BL/6 or HLA-DR4 transgenic mice were vaccinated subcutaneously 3 times at 14-day intervals with the different dosages (n=3-4 mice per group) to determine the optimal dose. Splenocyte recall responses to purified AgX protein were assessed *in vitro* by IFN-□ ELISPOT and ELISA assays 14 days following the final immunization to validate the optimal dose.

Results: The newly created mRNA vaccine is stable and easily synthesized. We determined that the GCP-encapsulated AgX mRNA vaccine was successfully taken up and expressed by phagocytes *in vivo* and by HEK-Blue cells *in vitro* by flow cytometry and by western blot, respectively. Mice vaccinated with as low as 5 ug of the mRNA vaccine were sufficiently able to mount a T cell memory response. Currently we are evaluating the protective efficacy of the GCP-AgX mRNA vaccine in C57BL/6 mice using a model pulmonary coccidioidomycosis.

Conclusions: Our findings demonstrate that our novel GCP-encapsulated AgX mRNA vaccine is both functional and generates memory T cell responses that we have previously shown correlate with protection in mice challenged with *Coccidioides*. Our future goal is to test the protective efficacy of this vaccine in both C57BL/6 and HLA-DR4 transgenic mice.

29 POSTER

ASYMPTOMATIC COCCIDIOIDAL MENINGITIS RELAPSE: A DEMON IN DISGUISE

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Introduction: Meningitis is recognized as the most serious form of disseminated coccidioidomycosis and if not treated is lethal. The Infectious Diseases Society of America's coccidioidal guideline recommends therapy for life. Relapse is common if treatment is stopped. Frequent analysis of Cerebral Spinal Fluid (CSF) is essential. This patient is a 43-year-old man with a two-decade history of coccidioidal meningitis who was diagnosed with asymptomatic relapsed coccidioidal meningitis.

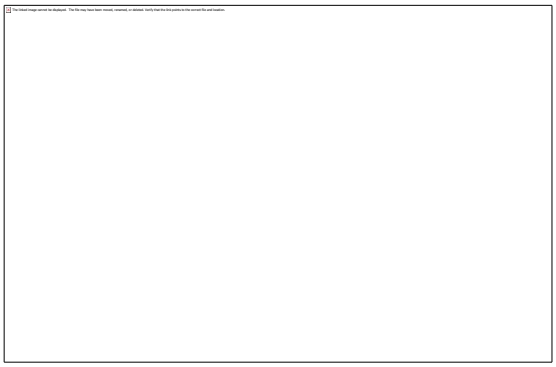
Methods: Retrospective case review approved by Kern Medical Institutional Review Board.

Literature Search: PubMed, Google Scholar for coccidioidal meningitis relapse, therapeutic drug monitoring, coccidioidomycosis.

Results/Case Presentation: A 43-year-old man had been diagnosed with coccoidal meningitis for two decades. His course was complicated by hydrocephalus, and therefore underwent placement of a ventriculoperitoneal (VP) shunt. His treatment was initiated on fluconazole 1000 mg daily. His care was complicated by multiple VP shunt revisions, the last episode was seven years prior.

Fluconazole levels were monitored at therapeutic goal levels of 40-80 µg/ml. CSFs were obtained periodically to monitor his response and showed minimal pleocytosis between eight to ten, normal protein and glucose, and coccidioidomycosis complement fixation (CF) titers of <1:1 repeatedly.

Periodically during the course of his care, he became nonadherent with medications and visits. He represented for a routine follow-up after a year and a half. At this visit, he admitted to being off of therapy for about seven months as he felt "great". He had a lumbar puncture done even though he was entirely asymptomatic. His CSF showed WBC of 261 µg/ml, predominately lymphocytic, glucose of 23 mg/dL, protein of 171 mg/dL, and CSF coccidioidomycosis CF titer of 1:8. Indicating a flagrant asymptomatic relapse. Medication compliance was reinforced.



Conclusion:

This case demonstrates that coccidioidal meningitis can relapse without any symptoms. This suggests the need for a standardized approach to monitoring disease even in asymptomatic patients that includes periodic evaluation of cerebral spinal fluid and therapeutic drug monitoring to reduce morbidity and mortality of relapsed cocciodioidal meningitis. Therapeutic drug monitoring can also assist in differentiating between treatment failure and non-adherence.

30 ORAL

COCCIDIOIDAL PULMONARY CAVITATION: A NEW AGE

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Abstract

INTRODUCTION: The majority of literature on cavitary pulmonary coccidioidomycosis is from 4 decades ago which was prior to the advent of triazoles and focused on surgical treatment. This observational study is a comprehensive retrospective study of pulmonary cavitary coccidioidomycosis from patients at Valley Fever Institute at Kern Medical over the last 12 years. This study aims to explore the spectrum of coccidioidal cavities and the evaluation and management of those cavities.

METHODS: IRB approved, retrospective review of electronic medical records of the Valley Fever Institute database was conducted. Demographics, comorbidities, types, and the number of cavities, complications, and medical and surgical treatment were gathered and compared to the literature. PubMed and Google Scholar were searched for cavitary pulmonary coccidioidomycosis.

RESULTS: Of the initially 276 identified patients, 137 met the inclusion criteria. This study found 52 (37.2%) patients with hemoptysis. One case (0.7%) required radiologic intervention to occlude the bleeding vessel, and one (0.7%) case of hemorrhage required right upper lobe lobectomy. No patients in this study required a Fogarty catheter.

Nine (6.6%) cases exhibited a ruptured cavity. Eight of those cases had initial chest tube placement, of which three (3/8, 37.5%) did not require surgical intervention. The remaining five (5/8, 62.5%) cases with an initial chest tube placement ultimately led to a thoracotomy or VATS (three (3/8, 37.5%) wedge resections, four (4/8, 50%) decortications, one (1/8, 12.5%) pleurodesis, and one (1/8, 12.5%) pneumonectomy).

Seven of 137 (5.1%) cases presented with a pleural effusion not associated with a cavity rupture. Five (3.7%) were due to primary coccidioidomycosis. The remaining two (1.5%) cases were attributed to heart failure. One of these two cases had a concurrent perinephric abscess with an associated exudative pulmonary effusion. This was the only cause of a non-coccidioidal exudative effusion in this study. Three of the coccidioidal effusions required therapeutic thoracentesis, and none required a chest tube or surgery.

When patients with insufficient data or dissemination were excluded, the mean duration of the initial antifungal treatment was found to be 563 days (n=80). In 35% (28/ 80) of them, a triazole was switched to another triazole for variable reasons, including treatment failure or side effects. The switch from one antifungal treatment to another was considered the end of the initial treatment.

CONCLUSION: Coccidioidal pulmonary cavitation remains a complex disease to evaluate and treat. In the present age of triazole therapy, indications and the need for surgery continue to decline. Further investigation needs to be conducted to evaluate medical therapy's efficacy and long-term outcomes.

Table 1. Demographic characteristics, concomitant dissemination sites if present, medical treatment, and complications of 137 patients with cavitary coccidioidal lesions.

Characteristic	Single Cavity (n = 91)	Multiple Cavities (n = 46)	Total (N = 137)
Demographics & Comorbidities			
Female	43(47.3%)	18 (39.1%)	61(44.5%)
Hispanic	61(67%)	37(80.4%)	64(46.7%)
Caucasian	22(24.2%)	3(6.5%)	25(18.2%)
Black	3(3.3%)	2 (4.3%)	5(3.6%)
Filipino	1(1.1%)	1 (2.2%)	2(1.5%)
Middle Eastern	1(1.1%)	0	1(0.7%)
Unknown Ethnicity	3(3.3%)	3(6.5%)	6(4.4%)
Diabetes Mellitus	56(61.5%)	34 (73.9%)	90(65.7%)
Smoking Tobacco	21(23.1%)	6(13%)	27(19.7%)
Documented COPD*	1(1.1%)	4 (8.7%)	5(3.6%)
Immunosuppressive medications	5(5.5%)	5 (10.9%)	10(7.3%)
Transplant	0	0	0
HIV	6(6.6%)	5(10.9%)	11(8.0%)
Presenting Manifestations			
Cough	43(47.3%)	12(26%)	55(40.1%)
Hemoptysis	26(28.6%)	12(26%)	38(27.7%)
Fever	18(19.8%)	11(23.9%)	29(21.2%)
Shortness of Breath	19(20.9%)	9(20%)	28(20.4%)
Night Sweats	18(19.8%)	4(8.7%)	22(16.1%)
Chest Pain	14(15.4%)	5(10.9%)	19(13.9%)
Weight Loss/Decreased Appetite	8(8.8%)	3(6.5%)	11(8%)
Fatigue	6(6.6%)	3(6.5%)	9(6.6%)
Location**			
Right Upper Lobe	31(34.1%)	16(34.8%)	47(32.8%)
Left Upper Lobe	26(28.6%)	18(39.1%)	44(32.1%)
Right Lower Lobe	18(19.8%)	5(10.9%)	23(16.8%)
Left Lower Lobe	13(14.3%)	4(8.7%)	17(12.4%)
Right Middle Lobe	3(3.3%)	3(6.5%)	6(4.4%)
Dissemination			
Osseous	9 (9.9%)	2 (4.3%)	11(8%)
CNS	6 (6.6%)	1 (2.2%)	7(5.1%)
Integumentary	3 (3.3%)	0	3 (2.2%)
Other	5 (5.5%)	2 (4.3%)	7(5.1%)
Total	23 (25.3%)	5 (10.9%)	28 (20.4%)
Treatment			
Fluconazole***	81 (89%)	41 (84.8%)	122 (89.1%)
Other Triazole***	1 (1.1%)	0	1 (0.7%)
Amphotericin***	4 (4.4%)	1 (2.2%)	5(3.6%)
None***	1 (1.1%)	2 (4.3%)	3(2.2%)
Unknown***	4 (4.4%)	2 (4.3%)	6(4.3%)
Surgery	4 (4.4%)	5 (10.9%)	9(6.6%)

Complications			
Hemoptysis	33 (36.3%)	18 (39.1%)	51(37.2%)
Superinfection	7 (7.7%)	5 (10.9%)	12(8.8%)
Pleural Effusion	1 (1.1%)	6 (13%)	7(5.1%)
Rupture, Empyema, or fistula	4 (4.4%)	5 (10.9%)	9(6.6%)
Pneumothorax	6 (6.6%)	4 (8.7%)	10(7.3%)
Fungal Ball****	3 (3.3%)	4 (8.7%)	7(5.1%)

*Chronic Obstructive Pulmonary Disease, **Multiple Cavities Location refers to the largest cavity, ***Initial medical treatment, ****Mass within cavity presumed to be a fungal ball.

31 ORAL

DRUG REPURPOSING FOR NOVEL ANTIFUNGALS THAT INHIBIT SPHERULE-PHASE GROWTH

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Introduction: Clinical isolates of *Coccidioides* have displayed a trend of increasing resistance to fluconazole. To ameliorate this, we need new antifungal drugs with novel targets. We have discovered several new compounds that inhibit spherule-phase growth by screening FDA-approved drugs from 3 chemical libraries. These drugs could potentially be repurposed for anti-*Coccidioides* therapies.

Materials and Methods: In a BSL-3 laboratory, arthroconidia of the fluconazole-resistant *C. posadasii* strain #C735 or the attenuated C. *posadasii* triple mutant strain (ΔT; Δcts2/Δard1/Δcts3) are cultured in Converse media for 24h to facilitate their conversion into spherules. Spherules were dispensed into each well of a drug plate containing up to 10µM of the library compounds then subject 24h later to an XTT assay for determining cell metabolic capacity as an indicator of growth. The metabolic capacity of spherules incubated with DMSO (drug vehicle; <1%) serves as a control. We have completed the screening of three drug libraries including the Broad Repurposing Hub (5440 drugs), the Prestwick (1520 drugs), and the Selleck L8200 Antiparasitic (220 drugs) libraries. Toxicity profiling was performed using the EPA CompTox batch search and validated with a human liver cell line, HepG2. A secondary phenotypic analysis using image flow was used to validate the 7 hits from the Halogenated Salicylanilide class to assess protonophore mechanism of action in *Coccidioides*. The drug-treated spherules were stained with calcofluor white which bind to the fungal cell wall, Mitotracker Green which measures mitochondrial mass, and Mitotracker Red CMXros which fluoresces when the mitochondrial membrane potential is in an oxidized state. Our partners at the Fungus Testing Lab assessed the efficacy Salicylanilides in the arthroconidia-phase in a panel of fluconazole-resistant *Coccidioides* clinical isolates, (n=5 C.i., n=1 C.p.) using CLSI standard methods. *In vivo* formulations were developed by the Date Lab.

Results: From the 7180 drugs screened 86 appeared as hits with >60% spherule growth inhibition. We also screened the Selleck library with the ΔT strain and compared the percent inhibitions against the values from C735. These values were comparable between the virulent C735 and the ΔT strain, suggesting the attenuated strain can be used as a surrogate for drug screening in a BSL2 laboratory. CompTox profiling showed that 24 of the compounds were strong *in vivo* candidates well characterized in terms of safety, toxicity, and physiochemical properties. Toxins, pesticides, and current antifungals were removed from the pool. Dose-response assays of the remaining 24 compounds included IC_{50} assays with C735 and CC_{50} assays with HepG2 to determine their respective antifungal efficacy and cytotoxic concentrations. The 7 Halogenated Salicylanilides tested displayed excellent potency compared to the non-halogenated backbone control. Image flow cytometry assessment of phenotypic changes to the mitochondria support the protonophore MOA of the Salicylanilides but suggest that there is an additional MOA since potency was not directly correlated to fluorescent intensity. 2 of the 7 Halogenated Salicylanilide drugs, Niclosamide (NIC) and its ethanolamine salt (NEN) display low IC_{50} from 1.5µM and inhibition activities against fluconazole-resistant *C. posadasii* and *C. immitis* strains. *In vivo* formulations of NIC and NEN with various excipients were efficacious *in vitro* and will move into *in vivo* testing.

Conclusion: Our 2-step drug screening method using a metabolic assay followed by a phenotypic image analysis has successfully identified 7 novel antifungal compounds from the halogenated salicylanilide class. These drugs are anthelmintics approved in either human or veterinary medicine and their oral formulations are generally considered to be low or non-toxic. In helminths, salicylanilides mechanism of action is to act as a protonophore that uncouples oxidative phosphorylation. We intend to repurpose these drugs for high-risk patients with prolonged pulmonary symptoms and disseminated disease.

32 ORAL

ASSOCIATION BETWEEN WILDFIRES AND COCCIDIOIDOMYCOSIS CASES IN CALIFORNIA, 2000-2018: A SYNTHETIC CONTROL ANALYSIS

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Abstract

Introduction The frequency and severity of wildfires in Western U.S. has increased over recent decades, motivating hypotheses that wildfires contribute to incidence of coccidioidomycosis, an emerging fungal disease in the Western U.S. While coccidioidomycosis outbreaks have occurred among wildland firefighters clearing brush, it remains unknown whether fires are associated with increased coccidioidomycosis incidence among the general population.

Methods We identified 19 wildfires occurring within California's highly endemic San Joaquin Valley between 2003 and 2015. Using geolocated surveillance records, we applied a synthetic control approach to estimate the effect of each wildfire on the incidence of coccidioidomycosis among residents that lived within a hexagonal buffer of 20 km radii surrounding the fire.

Results We did not detect excess cases due to wildfires in the 12 months (pooled estimated percent change in cases: 2.8%; 95% CI: -29.0, 85.2), 13-24 months (7.9%; 95% CI: -27.3, 113.9), or 25-36 months (17.4%; 95% CI: -25.1, 157.1) following a wildfire. When examined individually, we detected significant increases in incidence following three of the 19 wildfires that had relatively large adjacent populations and high annual transmission prior to the fire.

Discussion We find limited evidence of an association between coccidioidomycosis incidence and wildfires among the general population. The majority (15) of the fires did not demonstrate an effect on incidence in surrounding populations, although our results suggest that some fires may still have an effect. Longitudinal field studies examining presence of Coccidioides spp. in soil following wildfires are warranted.

33 POSTER

COCCIDIOIDES IGG-POSITIVE/IGM-NEGATIVE ELISA REACTIVITY AMONG ASYMPTOMATIC PATIENTS RESIDING IN UTAH, NEVADA, AND ARIZONA

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Abstract

INTRODUCTION Coccidioidomycosis (CM) is an infection caused by Coccidioides species, a dimorphic fungal pathogen with increasing endemicity in the Western United States. Serological testing in the form of IgG and IgM Enzyme Immunoassay (ELISA) has been a part of the clinical diagnostic standard for years. Traditional dogma suggests following infection with Coccidioides spp. and clearance of the pathogen, IgG does not remain elevated but instead returns to normal.

METHODS We searched all encounters in the Intermountain Healthcare electronic medical record from 2006-2015 for clinical data associated with CM. 788 potential cases of infection were reviewed manually – as a part of the review process, 114 (14.5%) patients with IgG+/IgM- by ELISA were found and excluded from the original dataset as they had no other accompanying clinical features of CM. We now describe this subset cohort to further understand the epidemiology and clinical characteristics of this group.

RESULTS Patients in the cohort were generally less healthy (median Charlson comorbidity index 3, IQR 2-6), elderly (median age 69.5 years, IQR 55-78 years), and overwhelmingly resided in areas of Utah known to be endemic for Coccidioides spp. including Washington and Iron Counties. Patients also appeared to actively rely on and access healthcare, indicated by high rates of hospitalization (>0 incidents 42-d 55.3%, 365-d 67.5%) and clinic visitation (>0 incidents 42-d 75.4%, 365-d 90.4%). Common conditions included chronic pulmonary disease (57.9%), congestive heart failure (36.8%), and diabetes mellitus (33.3%), among others. All-cause mortality was 7.0% at 42-d and 19.3% at 365-d.

CONCLUSIONS All patients in this cohort reside in areas of Utah, Arizona, and Nevada known to be endemic to Coccidioides spp. indicating that a false positive IgG is unlikely. Rather, IgG reactivity suggests either asymptomatic infection or a longer-lived adaptive immune response that may challenge the current paradigm of serology kinetics related to CM.

34 ORAL

THE RELATIONSHIP BETWEEN COCCIDIOIDES POSADASII AND BIOLOGICAL SOIL CRUSTS IN ARIZONA DESERT ECOSYSTEMS

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Abstract

Introduction: Increased dust emissions have been shown to be correlated with increased rates of Valley fever. To date, there is a lack of research on the role of environmental remediation and restoration of natural soil communities and the reduction of *Coccidioides* in the soil and dust. Biocrusts are diminutive communities composed of lichens, bryophytes, cyanobacteria, fungi, liverworts, and other microorganisms that live in and bind the top mineral soil layer and are common features of the dryland ecosystem. It has been shown that biocrust restoration can reduce global atmospheric dust emissions. Our overarching research objective is to determine if soil remediation with biocrust can reduce the occurrence of *Coccidioides* spp. in the air and/or soil by stabilizing the soil surface, reducing associated dust, and increasing below-ground microbial competition.

Methods: For a field trial, soil samples were collected in Arizona at the McDowell Sonoran Conservancy within and surrounding a biocrust restoration site during the spring, pre-monsoon season. Soils were collected using a garden trowel or kitchen spoon depending on soil depth and put directly into sterile 50-milliliter collection containers; trowels and spoons were sterilized with 10% bleach between soil samples. Twenty animal burrows were randomly picked surrounding the biocrust restoration experiment. Soils collected from inside animal burrows were taken from approximately 10 centimeters in depth (depending on size of burrow). Sixty soil samples were taken from surrounding biocrust restoration plots. Due to the lack of rodent burrows surrounding the restoration site and difficulty in detecting the pathogen via surface soil sampling, more soils were taken from biocrust plots to increase the chance of observing a trend. DNA extractions were done on the samples and then quantified with presence/absence real-time qPCR using CocciDX assay (Bowers JR et al. 2019; Saubolle MA et al. 2018; Litvintseva AP et al. 2014).

To determine the effect of biocrust and aerosolization of VF arthroconidia, 50mL vented conicals with desert soils were prepared with three different biocrust restoration treatments: full coverage, disturbed, and no coverage. After biocrust establishment, a syringe was used to inoculate the soil beneath the crust layer with 103 *C. posadasii* arthroconidia and incubated for 14 days at room temperature. A filter was placed above the soil surface layer during the 14-day incubation to capture aerosolized spores. DNA extractions were done on the filters and then quantified with presence/absence real-time qPCR using the CocciDX assay (Bowers JR et al. 2019; Saubolle MA et al. 2018; Litvintseva AP et al.2014).

Results: In the field trial, all biocrust restoration plots were found to be negative for *Coccidioides* spp. Two out of the twenty rodent burrows surrounding the restoration site were positive, indicating *Coccidioides* spp. is found in the area, and soils are more likely negative where biocrust is present. For the lab incubation experiment, all “no coverage” biocrust treatments resulted in a positive filter, showcasing *C. posadasii* aerosolization. Full biocrust coverage treatment showed a 50% decrease in C. posadasii positivity compared to the disturbed treatment group.

Conclusion: These findings provide evidence that restoration of natural dryland communities may be a useful tool to decrease *C. posadasii* burden in Arizona. Management practices that reduce the disruption of soil surfaces or restore disturbances with biocrusts may reduce the abundance of airborne soil pathogens, and thus reduce the spread of *C. posadasii*.

36 ORAL

COCCIDIOIDES ALONG RIPARIAN HABITATS IN THE SAN JOAQUIN VALLEY IN CALIFORNIA

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Abstract

Intro: *Coccidioides* spp., the fungi that cause coccidioidomycosis, are routinely found in soils collected from rodent burrows. In arid and semi-arid environments, rodent and small-mammal populations are found in higher abundance in riparian ecosystems than in adjacent upland ecosystems. Bakersfield and Coalinga, two cities in California, represent two population centers with riparian habitats running though or directly adjacent to their urban cores, each with a high annual incidence of Coccidioidomycosis.

Methods: From a total of 52 sites in the vicinity of Bakersfield and Coalinga, in the San Joaquin Valley in California, a region endemic to *Coccidioides* and high in coccidioidomycosis incidence, we surveyed 1030 rodent burrow soil samples for *Coccidioides* presence. We collected 825 samples from along two riparian habitats: the Kern River in Kern County and Los Gatos Creek in Fresno County, 205 samples from in and around Bakersfield, California and an additional 100 urban surface samples from Coalinga, California. Sampling along the Kern River occurred over one year at four timepoints, with a subset of samples spanning the entire period. All samples were tested for *Coccidioides* presence using the CocciEnv qPCR assay, and 68 rodent burrow soil samples (28 from the Kern River and 40 from Los Gatos Creek) were selected for sequencing of the internal transcribed (ITS2) region of fungal rDNA. Temporal patterns of *Coccidioides* presence were tested using logistic regression and associations with the soil fungal community were investigated though ordination, permutational analysis of variance and other multivariate statistical methods.

Results: *Coccidioides* was detected in 8.3% of samples from the Kern River and 24.5% of samples from Los Gatos Creek, though was not detected in any of the samples from in and around Bakersfield and in only one urban surface sample from Coalinga, California. *Coccidioides* was more commonly found closer to population centers, less commonly found on land showing less evidence of human habitation and never found in higher elevation, mountainous terrain. Coccidioides detection was geographically sporadic, being found at only 12 of 52 sites, with few clear patterns. However, *Coccidioides* detection was temporally consistent, being reliably detected at sites previously showing high levels of detection.

Conclusion: *Coccidioides* can be found abundantly and consistently in rodent burrow soils situated in urban and urban-adjacent riparian habitats in the San Joaquin Valley. The presence of *Coccidioides* may be associated with the density of rodent burrow populations, though further research is needed.

37 ORAL

LACK OF SHED SPREAD OR REVERSION OF VIRULENCE OF A ΔCPS1 IN VACCINATED DOGS

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Abstract

INTRODUCTON: Dogs are more highly susceptible to coccidioidomycosis than man and offer a One Health opportunity to evaluate the safety of the live avirulent Δcps1 candidate vaccine. For live vaccines microbial safety is evaluated, in required studies, based on compendial regulatory experimental guidance's conducted under stringent conditions. Requirements include backpassage/reversion to virulence (RTV) designs that endeavor to determine if the vaccine strain regains function and pathogenicity following serial passage/recovery of the master seed in the host species. In addition, Shed Spread (S/S) study designs follow the inoculation of the vaccine master seed to seronegative host species by the intended route and schedule, at multiples of the minimum immunizing dose. The design includes inclusion of cohoused naive non vaccinated sentinel controls. Monitoring comprises surveillance for seroconversion, clinical signs with repeated sampling/ culture of excretions (e.g. nasal/oropharyngeal swabs, urine, feces, any abscessed injection sites etc.). At termination, both inoculates and sentinel animals are assessed for pathological lesions, target tissues are cultured for presence of the vaccine strain, and seroconversion is evaluated to determine whether the vaccine has disseminated.

METHODS: In a pilot study that combined elements of both SS and RTV, 18 purpose bred Beagles (6-7 months old) were cohoused by gender. Seven male dogs were inoculated intratracheally with 56,000 Δcps1 spores and commingled with two sentinels. An additional seven female dogs were inoculated intratracheally with 115,000 Δcps1 spores and commingled with two sentinels. Dogs were monitored for 28 days for clinical signs, shedding of Δcps1 from nasal and oropharyngeal swabs, detection of lung lesions by digital radiographs, changes to hematology and serum biochemistry, and seroconversion (MiraVista, Indianapolis IN). On Day 28 post-exposure, dogs were euthanized. Gross necropsy was conducted of the lungs and other organs and pulmonary tissue fixed for histopathologic evaluation. Lung tissue samples were homogenized to recover Δcps1 by culture, with confirmation by PCR. IACUC Approval 1137.

RESULTS: All dogs lacked antibodies against Coccidioides prior to inoculation and remained healthy with no fever, clinical signs, lameness, or clinically significant hematological or serum biochemistry changes. Pre- and post-exposure lung radiographs were normal. Δcps1 was not cultured from nasal and oropharyngeal swabs collected weekly. At necropsy, 17/18 dogs had no abnormalities observed. One male dog had small lung lesions, but fungus was not isolated. Histopathology demonstrated rare, tiny perivascular/interstitial areas of pyogranulomatous or monocytic inflammation without visible organisms, possibly representing lung-associated lymphoid aggregates. Total culture of individual lung lobes from 16 dogs (4 sentinels, 7 male inoculates, and 5 female inoculates) had no growth of Δcps1. One Δcps1 colony was grown on GYE/hyg plates from 1 lobe of each of 2 female dogs inoculated with 115K viable spores and confirmed by PCR. No lymph nodes were culture positive. None of the dogs inoculated with Δcps1 or the cohoused sentinels seroconverted during the study.

CONCLUSIONS: These data demonstrate the Δcps1 Master Seed remained avirulent when inoculated into dogs by intratracheal nebulization of ≤115,000 spores. Additionally, this study failed to demonstrate risk of shed and spread of Δcps1 to sentinels when administered by the natural (respiratory) route of infection, A S/S study by the subcutaneous route is underway and will be reported at 67th Annual CSG Meeting.

40 ORAL

OCCUPATIONAL ENHANCED SURVEILLANCE OF COCCIDIOIDOMYCOSIS, MARICOPA COUNTY, 2018-2019

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Abstract

INTRODUCTION: Coccidioidomycosis is among the top three incident reportable diseases in Maricopa County; from 2018 to 2019, a yearly average of 6,461 cases was reported. Little is known about industrial/occupational risk factors for coccidioidomycosis in Maricopa County. Multiple Arizona courts have ruled that it is not possible for a worker to prove they contracted coccidioidomycosis while at work if their residence is in an endemic region, making Workers' Compensation data unhelpful. From July 2018 to June 2019, Maricopa County Department of Public Health (MCDPH) conducted enhanced surveillance to understand industry and occupational risk factors (industry refers to the kind of work performed by an individual's workplace and occupation refers to the kind of activity undertaken by an individual) that may be associated with testing positive for coccidioidomycosis.

METHODS:

Data
Reported coccidioidomycosis cases are entered into Arizona's statewide medical electronic disease surveillance system, MEDSIS. Cases are classified as "Confirmed" if there is positive coccidioidal serology (IgM or IgG reactivity by EIA or IMDF), positive culture, or positive histology.

MCDPH sought to interview 10% of confirmed coccidioidomycosis case-patients reported in the months preceding interviews. Individuals were randomly selected for interview using SAS v.9.1. If the case-patient refused interview or could not be reached by phone following 2 contact attempts over 2 days, the next case-patient was contacted for interview.

Enhanced Surveillance Investigation
Enhanced Surveillance Investigation Form
MCDPH worked with CDC's National Institute for Occupational Safety and Health (NIOSH) to develop and incorporate occupation- and industry-specific questions into the enhanced surveillance form. Questions from the American Community Survey were incorporated to standardize data collection and persons eligible for interview with available county workforce data.

Eligibility
Confirmed case-patients were eligible for randomization if reported in the month prior to randomization, 18 years of age or older, were not deceased, and had a phone number available. Individuals were excluded if they were not employed in the week prior to interview.

Industry and Occupation Data
NIOSH received de-identified industry and occupation case data. The NIOSH Industry and Occupation Coding System (NIOCCS) was used to auto-code occupation data to the 2010 Standard Occupation Classification (SOC) system, and industry data into the 2012 North American Industry Classification System (NAICS). All records, along with available case work data, were reviewed to ensure accuracy; occupation and industry codes were re-coded if necessary.

MCDPH cross-walked both the 2010 SOC codes and the 2012 NAICS codes into 2018 SOC codes and the 2017 NAICS codes, respectively, to align with the codes available in the American Community Survey (ACS) for analysis.

Denominators
The 2015-2019 ACS 5-year estimates were used for Maricopa County workforce data.

Analysis
Incident case rates were calculated using coccidioidomycosis cases within each industry sector and major occupation group and the corresponding industry sector and major occupation group in the 2015-2019 ACS data. A total of 18 major industry sectors were analyzed; 10 sectors were analyzed in groups of two in accordance with categories provided in ACS denominator data. Due to sample size and denominator limitations, only major occupation groups and industry sectors were analyzed. Twenty-one major occupation groups were analyzed. Industry sectors and major occupation groups with fewer than five observations were removed from analysis due to small numbers. Individuals in the armed forces were removed from the analysis as this group is not represented in ACS data. Calculations were conducted in SAS v.9.4.

RESULTS: All results are preliminary. Between June 2018 and May 2019, a total of 5,707 confirmed cases of coccidioidomycosis were reported to MCDPH. Of those reported, 3,254 (57.0%) were female, 2,442 (42.8%) were male, and 11 (0.2%) were unknown. Ages ranged from 0 to 99 years of age (median 55 years). Of 5,348 (93.7%) cases meeting initial interview eligibility criteria, 599 (11.2%) interviews were attempted. One hundred eighty-four (30.7%) persons were successfully interviewed, 116 (19.4%) were excluded due to not working the week prior, 252 (42.2%) were lost to follow-up, and 47 (7.8%) refused. Industry or occupation data were available for 182 (98.9%) cases. Of these, 3 cases had insufficient data to determine industry sector, and 5 cases had insufficient data to determine a major occupation group. The industry sectors with the highest coccidioidomycosis rates were educational services and health care (NAICS 61 and 62, respectively; 12.4 cases per 100,000 workers), construction (NAICS 23; 12.3 cases per 100,000 employed population), and wholesale trade (NAICS 42; 9.5 cases per 100,000 workers). The major occupation groups with the highest coccidioidomycosis rates were computer and mathematical (SOC 15; 13.8 cases per 100,000 workers), healthcare practitioners (SOC 29; 12.5 cases per 100,000 workers), and office and administrative support (SOC 43; 10.9 cases per 100,000 workers).

CONCLUSION: Our preliminary findings indicate certain occupational groups and industry sectors may be at increased risk of coccidioidomycosis infection, however interpretation of the occupation data is challenging without additional data regarding outdoor exposure. Confounding factors could include variation by employment category in access to healthcare, testing, general outdoor exposure, or availability for interview. MCDPH plans to implement additional enhanced surveillance activities by occupational groups and industry sectors in 2023.

41 POSTER

SUBA-ITRACONAZOLE VERSUS CONVENTIONAL ITRACONAZOLE IN THE TREATMENT OF COCCIDIOIDOMYCOSIS: A SUBGROUP ANALYSIS OF MSG-15

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INTRODUCTION: Itraconazole (ITZ) is an effective agent in the treatment of fungal disease including coccidioidomycosis. However, ITZ is limited by drug tolerability, food requirements, and variability in serum concentrations. A new formulation, SUBA-itraconazole (for "super bioavailability"; S-ITZ), addresses the limitations of conventional ITZ formulations.

METHODS: This was an open label randomized trial conducted to evaluate the efficacy and safety of SUBA-itraconazole compared to conventional itraconazole (c-itra) in the treatment of endemic fungal infections. Responses at days 42 and 180 were determined by an independent data review committee.

RESULTS: Eighty-eight patients were enrolled for IFD (SUBA-itra, n=42; c-itra, n=46) caused by Histoplasma (n=51), Blastomyces (n=18), Coccidioides (n=13), and Sporothrix (n=6). In the Coccidioides group 9 were randomized to SUBA-itra and 4 to c-itra. Clinical responses for SUBA-itra at day 42 were success in 5 coccidioidomycosis patients, failure in 3, with 1 unevaluable. Clinical responses for c-itra at day 42 were satisfactory in 2, with unevaluable status in 2. At day 180 a clinical response in SUBA-itra treated patients was observed in 5, with failure in 3 and unevaluable in 1. At day 180 c-itra were satisfactory in 1 patient and unavailable in 3. There was no difference in tolerability at days 14, 28, 42, 84 or 180. Fewer adverse events and serious adverse events were seen in the SUBA-itra treated patients (74% and 10% respectively) compared to those receiving c-itra (87% and 26%).

Clinical and Mycologic Outcomes in all Endemic Mycoses at days 42 and 180

Outcome	Clinical D42	Mycologic D42	Clinical D180	Mycologic D180
SUBA-Itra N=42				
Success	29 (69%)	19 (45%)	25 (60%)	17 (40%)
Failure	8 (19%)	9 (21%)	6 (14%)	7 (17%)
Unevaluable	5 (12%)	14 (33%)	11 (26%)	18 (43%)
c-Itra N=46				
Success	31 (67%)	22 (48%)	30 (65%)	22 (48%)
Failure	7 (15%)	14 (30%)	5 (11%)	9 20%)
Unevaluable	8 (17%)	10 (22%)	11 (24%)	15 (33%)

SUBA-itra, SUBA itraconazole; c-itra, conventional itraconazole

Conclusions. SUBA-itraconazole was well-tolerated and efficacious in the treatment of endemic fungi, including coccidioidomycosis, with a favorable safety profile compared to conventional itraconazole.

42 POSTER

MIRAVISTA DIAGNOSTICS SECOND-GENERATION *COCCIDIOIDES* ANTIBODY DETECTION ENZYME IMMUNOASSAY

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Introduction. Antibody detection is the most common method used for diagnosing coccidioidomycosis. MiraVista Diagnostics (MVD) introduced an anti-Coccidioides antibody enzyme immunoassay (EIA) in 2017 (Malo J. J Clin Micro). This assay has been modified to improve turnaround time and clinical accuracy.

Methods. This is a retrospective study evaluating a subset of specimens from Valleywise Health Medical Center (VHMC) collected between January 2013 and May 2017 (Kassis, C. CID 2020) in a 2nd gen antibody EIA. Cases had confirmed coccidioidomycosis based on chart review and positive serology while controls lacked a diagnosis of coccidioidomycosis. Here we report validation testing of the MVD 2nd gen EIA using specimens from VHMC study and compare these results to those using the 1st gen assay (Malo 2017).

Results. Intra-run precision for IgG antibody ranged from 8.1% CV for a high positive sample to 10.9% CV for a low positive sample and inter-run from 14.2% CV for a high positive sample to 17.9% CV for a low positive sample. Sensitivity and specificity of the 2nd gen assay was higher than the 1st gen assay and markedly higher than ID or CF ($p<0.01$). The assay performance time was also reduced by >2-fold permitting same day reporting of results.

Conclusions. The 2nd gen MVD EIA offers a rapid, accurate and reproducible method for assessing antibody response in coccidioidomycosis. The assay is more sensitive than ID or CF and high intra-assay and inter-assay precision allows for comparison of results between assays. The 2nd gen MVD EIA will be available for use in June 2023.

Assay-IgG	**Sensitivity**	**Specificity**
ID-IgG[1]	53.4% (55/103)	98.8% (162/164)
CF[1]	64.5% (40/62)	100% (52/52)[2]
MVD 1st gen EIA[1]	87% (90/103)	90% (153/170)
MVD 2nd gen EIA	92.0% (46/50)	95% (95/100)
Assay-IgM	**Sensitivity**	**Specificity**
ID-IgM[1]	33.0% (34/103)	100% (164/164)
MVD 1st gen EIA[1]	61.2% (63/103)	95.3% (162/170)
MVD 2nd gen EIA	46.0% (23/50)	99.0% (99/100)

[1]Malo, J. JCM 2017

[2]Kassis, C. CID 2020

43 ORAL

RARE LOSS OF FUNCTION VARIANTS IDENTIFIED IN PUTATIVE COCCIDIOIDOMYCOSIS RISK GENES IN NOVEL WHOLE GENOME SEQUENCED COHORT FROM THE VALLEY FEVER INSTITUTE

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Abstract

INTRODUCTION. Coccidioidomycosis is a fungal infection caused by the dimorphic fungus Coccidioides immitis or Coccidioides posadasii. Infected individuals display a wide range of severity of infection, ranging from asymptomatic, respiratory symptoms that resolve without treatment (uncomplicated valley fever, UVF), and individuals with severe presentations ranging from pulmonary only infection on treatment (POOT) to rare disseminated coccidioidomycosis (DCM). Previous studies in mice and humans have identified potential genes and genetic risk loci contributing to the more severe forms of coccidioidomycosis infection.

METHODS. To explore the genetic component of dissemination risk, our study recruited individuals with a history of coccidioidomycosis from UC Davis and the Valley Fever Institute (VFI). Whole genome sequencing (WGS) was performed for a cohort of 87 individuals from the VFI with DCM (n=49), POOT (n=27), or UVF (n=11) levels of disease severity. Potential loss of function (LoF) variants were identified using variant effet predictor (VEP) which predicted protein-level consequences. Likely LoF variants were filtered based on pathogenicity predictions from PolyPhen, SIFT, and CADD Phred. We looked for likely LoF variants in previously published fungal infection risk genes, including STAT3, CLEC7A, PLCG2, DUOX1, and DUOXA1. RNA sequencing (RNA-seq) was performed for a preliminary cohort of 39 individuals (23 DCM, 8 POOT, and 8 UVF). In addition to differential expression (DE) analysis carried out between UVF and DCM patients, significant differences in gene expression for likely LoF variant carriers were assessed with DESeq2.

RESULTS. We identified 15 patients harboring previously reported coccidioidomycosis risk variants from CLEC7A (rs16910526 and rs16910527) and PLCG2 (rs1143687). 93% (14/15) subjects with these variants had a more severe form of coccidioidomycosis (5 POOT, 9 DCM). We were able to identify an additional 10 likely LoF variants in 14 patients from STAT3, CLEC7A, PLCG2, and DUOX1. We also identified eight cases with rare and common variants that are predicted to alter splicing of exon 3 of CLEC7A. Mouse models have previously shown that exon 3 skipping increased the likelihood of coccidioidomycosis infection. Using the matched RNA-sequencing data, we sought to determine whether any of the putative variants identified had common effects on the transcriptome. Of the 23 patients with at least one likely LoF variant in a known risk gene, 12 patients had RNA-seq data. We identified three samples with PLCG2 likely LoF variants that had significant DE of 15 genes, including upregulation of genes relevant to immune regulation like ARG2, MUC6, and RIOK3 ($p<0.05$).

CONCLUSIONS. We have identified putative risk variants and novel rare variants in immune-function genes across our cohort of coccidioidomycosis patients. Additional matched RNA-sequencing data will allow for a more comprehensive and well-powered assessment of these variants' effects across these patients. One caveat to this study is that the cohort has few UVF patients relative to DCM/POOT cases and is underpowered to assess pathogenicity of even previously reported alleles in the absence of experimental data or large-scale biobank data. While our study has identified previously reported risk variants in our cohort, the rates of severe disease in these patients harboring rare mutations is not significantly different from rates of severe disease across the entire cohort. Improved power and orthogonal lines of evidence will allow us to more definitively establish the role and effect of coccidioidomycosis severity risk variants.

44 ORAL

IDENTITY-BY-DESCENT SEGMENTS AND THEIR RELATIONSHIP TO DISSEMINATED COCCIDIOIDOMYCOSIS

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INTRODUCTION: Coccidioidomycosis is a fungal infection endemic to the Americas. Infections can range from asymptomatic to pneumonia and in a small fraction of patients more severe forms with dissemination to the brain, bone, skin or other organs. Disseminated coccidioidomycosis (DCM) is a significant cause of morbidity and mortality. The risk factors for disseminated coccidioidomycosis remain unclear. Epidemiological studies over the past 70 years have shown an increased risk of disseminated disease in individuals who identify as African American, Latino, Filipino or Native American as well as increased risk of DCM in men compared with women. To address the genetic and biological basis of severe coccidioidomycosis outcomes, we used genetic mapping tools to identify regions of the genome that are identical-by-descent (IBD) in individuals who have more severe presentation of coccidioidomycosis. These regions that are shared among a subset of individuals with coccidioidomycosis likely harbor variants that increase risk of severe disease.
METHODS: In this study we use a total cohort of 555 individuals, 468 individuals from the UC-Davis Center for Valley Fever, and 87 individuals from the Valley Fever Institute at Kern Medical (VFI). In total, we have 245 cases and 310 controls. Cases were defined as those individuals who had either disseminated coccidioidomycosis (DCM) or pulmonary only on treatment (POOT). Controls were individuals who were diagnosed with coccidioidomycosis but fully recovered without treatment. Using the 87 genomes of our dataset, we ran an IBD segment detection algorithm called iLASH followed by a clustering algorithm called DASH to detect IBD segments that may be related to DCM risk. We filtered our IBD clusters to identify high quality clusters with multiple individuals and used heuristic parameters to identify segments which appeared enriched in severe coccidioidomycosis individuals.
RESULTS: We identified IBD segments in multiple regions of the genome that may be associated with risk of severe coccidioidomycosis. One IBD segment was found to be present across 6 cases and contains the gene neurogenic locus notch homolog protein 2 (NOTCH2), which is an established immunodeficiency gene involved with function and differentiation of immune cells. There are also additional segments associated with severity of coccidioidomycosis that we are continuing to investigate the potential biological role of in response to infection.
CONCLUSION: This unique approach allowed us to identify genetic regions that are shared among several individuals that have a history of severe forms of coccidioidomycosis. Our approach suggests that there is significant heterogeneity in the genetic risk factors associated with disease. These segments give us the potential to leverage genetic biomarkers to identify individuals at the highest risk for DCM and potentially tailor targeted treatments. Validation of these findings in other data sets are ongoing to understand the full genetic architecture of coccidioidomycosis risk.

45 ORAL

PATHOGENESIS OF *COCCIDIOIDES POSADASII* AND SARS-COV-2 CO-INFECTION IN THE K18-HUMAN ACE2 TRANSGENIC MOUSE MODEL

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Introduction: Early reports showed that patients with COVID-19 who had a previous diagnosis of Coccidioidomycosis (Valley fever, VF) who had cleared VF months and years prior had re-emergence of VF after COVID-19. However, total numbers of annual cases reported to CDC did not appear to rise dramatically. We therefore investigated serial infection of Coccidioides (Cocci) and variants of SARS-CoV2 in an K18-hACE2 transgenic mouse to assess the impact on disease outcomes.

Methods: We intranasally challenged mice sequentially with sub-lethal doses of 100 plaque forming units (PFUs) per mouse of SARS-CoV-2 (variants WA-1, Delta, Omicron BA1) and 24 hours later with 100 arthroconidia per mouse of a low virulence *Coccidioides posadasii* strain 1038 (Shubitz et al. 2021) and vice versa. Mice were monitored for 21 days post infection and euthanized if 20% of initial body weight was lost or a disease score < 6. Lungs, brain, and spleen were extracted and cultured to assess fungal burden and half of the lung was saved for histopathology. Kaplan-Meier survival curves with a log-rank test were used to detect statistical differences. To assess pathogenesis, we repeated the above experiment but sacrificed a subset of mice on days 1, 3, 5, and 6 post-infection. Lung homogenates were plated on 2xGYE with antibiotics to estimate fungal burden using CFUs and viral burden was assessed with one step reverse transcriptase real time qPCR at each time point (Dong et al. 2022). Serum was collected at each time point to measure differences in systemic cytokine/chemokine responses using a 26-plex cytokine assay (ThermoFisher) measure on a MagPix instrument (Luminex® Corporation, Austin, TX, USA with Luminex® xPONENT® 3.0 software). Animal work under NAU IACUC protocol # 21-025.

Results: We examined differences in disease outcome between the co-infected groups and groups that only received a primary infection. Co-infected groups had a more severe disease progression as well as decreased survival dependent on SARS-CoV-2 variant (WA-1, Delta, Omicron) and infection timing (CoV2 first, Cocci second or vice versa). The groups that were infected with the virus first had decreased survival, increased morbidity, and increased fungal and viral burdens. Additionally, virus first groups had an increase in circulating pro-inflammatory cytokines.

Conclusion: This is the first in vivo investigation of SARS-CoV-2 and Coccidioides co-infection in mice. Because of the increased severity of disease, we propose that SARS-CoV-2 can complicate coccidioidomycosis progression in endemic regions.

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46 ORAL

DEVELOPMENT AND EVALUATION OF A RAPID 10-MINUTE ASSAY TO DETECT ANTI-COCCIDIOIDAL ANTIBODIES

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Abstract

Introduction: The patient antibody response to infection with Coccidioides is often used for the diagnosis and monitoring of coccidioidomycosis, or valley fever (VF). In certain clinical settings there is a need for a rapid test to distinguish VF from viral and bacterial community acquired pneumonia (CAP) so appropriate treatment can be initiated. Such a rapid test would also be helpful for ongoing care of patients with VF. However, current antibody tests are run in clinical or reference laboratories, so the results are not integral to the visit with the healthcare provider. Here, we present a novel rapid antibody test for VF that is species agnostic and can provide an answer in 10-15 minutes.

Methods: Patient antibody responses to a single antigen, chitinase-1 (CTS1), were investigated using an indirect EIA. A total of 323 serum samples were tested for the presence of CTS1 IgG antibodies (n = 116 positive by EIA, ID and CF; n = 92 with discordant results by EIA, ID and CF; n = 86 endemic samples negative by EIA, ID and CF; n = 29 nonendemic/ other mycoses samples). Some of the endemic negative samples included patients with non-VF CAP. A subset of 143 serum samples were subsequently tested by our CTS1 LFA and IMMY's sōna Coccidioides antibody LFA. The agreement between sōna and ID/CF diagnostic assays was evaluated using sensitivity and specificity analyses. Because of the quantitative nature of our CTS1 LFA, sensitivity and specificity were evaluated using receiver operating characteristic (ROC) analysis. Human sera were collected under an Arizona State University institutional review board (IRB)-approved protocol no. 0601000548 and Mayo Clinic IRB protocol no. 12-000965.

Results: Using a CTS1 indirect EIA, we found that 115 of 116 (99.1%) patients with positive ID *and* CF agreement produce antibodies to CTS1. Expanding this to include any patient with a positive ID *and/or* CF result, 165 of 178 (92.7%) patients had anti-CTS1 antibodies. We then developed a CTS1-based lateral flow assay that is species agnostic and can be quantitative with the use of an LFA reader. Of the 143 patients tested by LFA, 113 patients (who were ID *and/or* CF positive n = 70; control samples n = 43) were included in the ROC analysis to determine sensitivity and specificity. With a cutoff of 46,000 density units, the CTS1 LFA has a sensitivity of 92.9% and specificity of 97.7%. The same patients were run on IMMY's sōna LFA which was 64.3% sensitive and 79.1% specific. Test line densities for our CTS1 LFA correlated in a linear manner with the lab-reported CF titer.

Conclusion: A 10-minute point-of-care test for the rapid diagnosis of VF could help healthcare providers make decisions in real time, usually while the patient is present. In high prevalence settings, it is vital to rule-in or rule-out a diagnosis of VF quickly (<20 minutes) so that patients may receive appropriate treatment–and importantly avoid inappropriate antibacterial therapy, which can contribute to the problem of antibacterial resistance. For patients with chronic VF infection, titers could be rapidly monitored such that a healthcare provider could know the patient's titer during the visit and adjust anti-fungal therapy in conjunction with the clinical presentation of the patient. This feature of our rapid test improves the efficiency of healthcare delivery.

47 ORAL

SAFETY AND EFFICACY OF Δ*cps1* VACCINE IN MICE WITH PRIMARY IMMUNODEFECIENCES

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Abstract

Introduction. While the majority of Coccidioides clinical cases are uncomplicated and require minimal intervention, a subset result in disseminated coccidioidomycosis (DCM). There are a variety of risk factors for DCM including AIDS, pregnancy, race/ethnicity, and of particular interest immunosuppression by either exogenous immunosuppression or a variety of known primary immunodeficiencies (PID). Patients with DCM have been identified with PID in a variety of pathways, including IL-12/IFN-gamma, STAT3, STAT4, Dectin-1, and Duox-1. Here we test the ability of our live attenuated Δ*cps1*vaccine to not only be tolerated by mice with PID but also impart protection from lethal challenge.

Methods. Wild Type (WT) or mice with a variety of PID were inoculated with ~10,000 Δ*cps1* spores via the subcutaneous route 2 weeks apart. Four weeks after the booster mice were challenged intranasally with 50-100 spores of strain Silveira. Mice were monitored for disease progression and survival. 14 days after the challenge surviving mice were euthanized and fungal burden in the lung and spleen was determined by serial dilution on 2x glucose-yeast extract (GYE) plates.

Results. We vaccinated the following PID mouse strains; *Stat4* KO, *Ifngr* KO, *Dectin-1* KO, *Rag-1* KO and *Stat3*-(V426) along with B6 controls. All mutant mice, as well as the B6 controls, tolerated both administrations of the vaccine with no complications. After challenge with strain Silveira all PID strains except for the *Rag-1* KO had significantly decreased lung and spleen fungal burdens compared to unvaccinated control. The *Rag-1* KO mice, though tolerant of the vaccination, had equivalent fungal burdens indicating the adaptive immune response in total is needed for effective protection. When comparing spleen fungal burdens, unvaccinated B6 mice had 100% dissemination to the spleen. The PID mice, in total, had detectable dissemination in 42% of mice (12% in B6 mice). Even when splenic dissemination was observed the burdens were significantly lower than in unvaccinated mice.

Conclusions. We have previously shown extensive and durable protection in WT mice using the Δ*cps1* vaccine. Here we highlight that the Δ*cps1* vaccine strain is extremely well tolerated in mice with PID. Additionally, these mice that have PID that would predispose them to DCM are able to be protected with the Δ*cps1* vaccine indicating the protection is multi-factorial. The tolerance of the vaccine in PID mice as well as the strong protection indicate this vaccine is safe and may provide protect in individuals with predisposition for DCM.

48 POSTER

ASSESSMENT OF THE UTILITY OF A HIGHLY REPEATED GENOME REGION TO CHARACTERIZE WITHIN SAMPLE *COCCIDIOIDES* POPULATIONS

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Abstract

Introduction: Environmental detection of *Coccidioides* has focused on utilizing real-time PCR for presence/absence detection. However, this technique, although common practice, is not equipped to efficiently detect and characterize mixed populations of *Coccidioides* spp. or subspecies within a sample. This question becomes especially important when analyzing samples with higher likelihoods of mixed populations, such as air sampling where arthroconidia are being aerosolized from multiple locations across space. Therefore, to further understand within sample populations, we must turn to a new technique that allows for efficient mixed population characterization.

Methods: We assess the applicability of an amplicon sequencing assay targeting a highly repeated genome region, commonly targeted by real-time PCR assays (CocciEnv, etc.), to identify and characterize mixed populations from samples with a single population of *Coccidioides*. We utilize an in-silico analysis of published genomes to characterize the diversity of the repeated region within clinical isolates and between isolates.

Results: Utilizing an in-silico PCR analysis of the CocciEnv assay the copy number was highly variable across genomes, with an average of 112 (range: 40-201) amplicon copies in *C. immitis* and 64 (range: 29-113) copies in *C. posadassi*.

Conclusions: Utilizing the variable region may allow individual strains or subgroups to be identified, thereby acting as a target for a population analysis associated with complex samples (e.g., environmental samples).

50 ORAL

EX VIVO RELEASE OF INTERFERON-γ (IFN-γ) AND INTERLEUKIN-2 (IL-2) IN RESPONSE TO WHOLE BLOOD INCUBATION WITH RECOMBINANT COCCIDIOIDAL ANTIGENS

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Abstract

INTRODUCTION: Within endemic regions, it is common to screen individuals prior to organ transplantation or the initiation of immunosuppressive therapy for the presence of anti-Coccidioides antibodies. Such assessment may indicate possible active infection and signal the need for antifungal prophylaxis but doesn't address prior quiescent infection. This study seeks to establish an ex vivo cytokine release assay using recombinant Coccidioides antigens using IFN-γ or IL-2 as an endpoint, to assess past infection and cellular immunity, with a goal of improving risk stratification, prophylaxis strategies, and future need for vaccination.

METHODS: Three recombinant coccidioidal antigens were expressed and isolated from *E. coli* or *Uncinocarpus reesii*. Contaminating endotoxin was removed using commercially purchased resin followed by ensuring that endotoxin levels were below the detection limit using the LAL method (<0.1EU). Protein concentration was determined using the BCA method and normalized by converting concentrations to molarity using molecular weight. Based on preliminary results, combinations of antigens were also used in a subset of subjects. Whole blood was collected from individuals with clinically resolved (latent) coccidioidomycosis from Arizona (IRB 2105781507) and stimulated using T27K, recombinant antigens, positive control (SEB or PHA) or media alone for 24 hours at 37°C with 5% CO2. Individuals with acute illness or medications known to suppress the cellular immune response were excluded. The concentration of supernatant IFN-γ and IL-2 was quantified by ELISA (BioLegend). Data are presented as concentrations of samples that were antigen-stimulated minus control. Never-infected healthy individuals (by personal history) from the endemic area (Phoenix, AZ) were also recruited.

RESULTS: The viability of whole blood stimulation for cytokine production was verified using the positive control SEB. T27k and three recombinant antigens stimulated the release of both IFN-γ and IL-2 in whole blood. IFN-γ minus nil stimulation of the three recombinant antigens ranged from an average of 96.6±118.9 pg/mL to 9,864.8±19,269.7 pg/mL. IL-2 minus nil stimulation ranged from an average of 85.0±68.4 pg/mL to 1,567.6±942.7 pg/mL. Antigen B showed a higher capacity to stimulate IL-2 whereas other antigens preferentially stimulated IFN-γ. For all antigens, a significant difference between the average antigen stimulation of IL-2 and nil control was observed ($p < .0002$ for all antigens). There was modest correlation between IL-2 and IFN-γ (Kendall's Tau-b correlation .009, .127, -.289) for the three recombinant antigens. A statistically significant correlation between IL-2 and IFN-γ for T27k was observed (Kendall's Tau-b correlation .402, $p=.04$). In one subject, response to antigens was robust greater than 9 years post infection.

CONCLUSIONS: These results demonstrate the feasibility of using recombinant coccidioidal antigens to induce cytokine production from individuals with prior coccidioidal infection. Either IFN-γ or IL-2 served as reliable cytokine markers. The use of recombinant antigens allows for more reproducible antigen production and standardized processes for use in an *ex vivo* cytokine release assay.

We acknowledge the collaborations of Banner-University Medical Centers in Tucson and Phoenix and the Valley Fever Center for Excellence

51 ORAL

COCCIWATCH: UNDERSTANDING THE EXPOSURE RISK OF AEROSOLIZED COCCIDIOIDES IN A VALLEY FEVER ENDEMIC METROPOLIS

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Abstract

Introduction: Coccidioides, the causative agent of Valley fever, is endemic to the arid climates of the western hemisphere. Although Coccidioides has been an established pathogen for 120 years, little is known about when and where infectious Coccidioides arthroconidia are present within the ambient air in endemic regions. Long-term air sampling programs provide a means to investigate these characteristics across space and time.

CocciWatch: Understanding the exposure risk of aerosolized Coccidioides in a Valley fever endemic metropolisMethods: We collected air filters daily from 11 air sampling sites across the Phoenix, AZ metropolitan area spanning an 18-month period. Air filters were extracted and Coccidioides was detected using real-time PCR. Environmental covariates were paired with sampling data to investigate drivers of prevalence utilizing statistical models.

Results: Overall, arthroconidia prevalence was highly variable across space and time, with no obvious spatial or temporal characteristics. Several high prevalence periods were identified at sites; however, these high prevalence periods did not have obvious spatial or temporal associations. Comparing these data to weather and environmental data, high wind gusts and temperature were positively associated with Coccidioides detection, while soil moisture was negatively associated with Coccidioides detection.

Conclusions: These data provide critical insights into the drivers, frequency, and distribution of airborne arthroconidia within the ambient air and the associated risk that is present across space and time in a highly endemic locale.

52 ORAL

DECTIN-1: A PARTIAL EXPLANATION FOR SEVERE COCCIDIOIDOMYCOSIS IN DOGS?

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Abstract

Introduction: DECTIN-1, encoded by *CLEC7A*, is a C-type lectin receptor critical for β-glucan recognition on fungal cell walls. Increased Coccidioides susceptibility in C57BL/6 mice is caused by a *Clec7a* splice mutation causing deletion of the extracellular stalk region placing the C-type lectin recognition domain (CTLD) closer to the cell surface[1]. Recent human work demonstrated common variants in the DECTIN-1 signaling pathway causing decreased β-glucan recognition and decreased TNFα production are overrepresented in patients with DCM and chronic pulmonary coccidioidomycosis[2].

Risk of *Coccidioides* infection for dogs living in the endemic region have been estimated to be 11-17% per year[3]; clinical presentations resemble those seen in humans including asymptomatic, contained pulmonary, and disseminated disease. While disseminated coccidioidomycosis (DCM) occurs in roughly 8% of symptomatic humans[4], 25% of symptomatic dogs have disseminated disease[5], frequently with osteomyelitis and central nervous system symptoms.

Methods: In silico analysis, RT-PCR, and sequencing was performed for dog *CLEC7A*/DECTIN-1.

Results: Hypothesizing DECTIN-1 defects could contribute to the increased dissemination seen in dogs we compared human and dog DECTIN-1 proteins using BLAST. Surprisingly, while the CTLD is 78% identical to human, the bioinformatically predicted dog DECTIN-1 contained a 46 amino acid gap corresponding to the exon 3 stalk region similar to the protein found in C57BL/6 mice. In silico examination of the flanking splice regions reveals a very weak splice site at the intron 2/exon 3 junction. Consistent with these findings, RT-PCR of *CLEC7A* cDNA from 5 individual dogs produced both expected size cDNA as well as a significant proportion of Δexon 3. Sequencing of the RT-PCR products defined two species of cDNA, the first was the predicted Δexon 3 while the second included exon 3 but also utilized a cryptic splice site 48 bases into intron 2. Critically, usage of this cryptic splice site introduces an in-frame stop codon. Sequencing 24 clones from one dog identified 16/24 with Δexon 3 and 8/16 containing exon 3 and the intron 2 in-frame stop codon due to utilization of the cryptic splice site.

Conclusion: Altered *CLEC7A* splicing may lead to insufficient DECTIN-1 function impacting *Coccidioides* detection in domestic dogs. Diminished initial fungal recognition and downstream response may contribute to the high rate of dissemination seen among dogs, similar to observations in mice and humans.

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54 POSTER

CUTANEOUS COCCIDIODOMYCOSIS - A GREAT IMITATOR

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Objective: Cutaneous Coccidioidomycosis (CC) infection can present with a wide variety of clinical presentations and is well known as a "great imitator". Failure to recognize the various manifestations of CC may lead to delay in diagnosis and treatment. We conducted a study of CC cases in our center to better understand physician diagnostic practices.

Methods: We performed a retrospective analysis of patients with CC in a large referral center in central valley, California, from 2010 to 2022 using the ICD9 and ICD10 codes for coccidioidomycosis and CC. Data pertinent to CC were collected, including demographics, underlying medical conditions, clinical, laboratory, and imaging findings, as well as follow-up treatment course and outcomes.

Results: We identified forty patients with CC during the study period. The mean age of the study population was 43 years (with standard deviation of 14.08). Among these, 60% were men and 40% women (table 1). The appearance of the lesions varied from ulcers, plaques, nodules and blisters to cellulitis and abscesses. The most common site of CC lesions was in the lower extremities (42.5%), followed by upper extremities (30%), chest and abdomen, head and neck (25 % each). Out of all these, 13 (32.5%) presented with lesions in multiple locations. Only 40% cases had biopsy available, and culture was available in 67.5% out of which 92.5% were positive. Serology was positive for IgG in 82.5% and IgM 35%, 5% did not have any serology done. Lung was the most common “other” organ involved.

More than 70% of these cases presented during the months of August through February. As previously noted in literature the number of cases peaked during the years of 2016-2017. The time from the onset of skin symptoms to the diagnoses either through culture or serology was on an average 8-16 weeks. Only 22.5% of the 40 cases were diagnosed as CC and 15% were diagnosed as erythema nodosum. The rest were diagnosed initially as bacterial cellulitis in 37.5%, tinea in 7.5% and others in 12.5 %. Among the identified cases, 95% received treatment with antifungal agents. The most common antifungal used was fluconazole in 78.9%, followed by itraconazole (15.7%) and voriconazole (5.26%). There was resolution of the cutaneous lesions in all patients with antifungal treatment.

Conclusions: Delays in diagnosis of CC are common even in endemic areas with high prevalence, as the appearance of the lesions may be varied and imitate the presentation of bacterial cellulitis, tinea and cysts. The mean time of diagnosis from onset of symptoms on an average was 12 weeks (8-16weeks) in our study with 75% cases initially misdiagnosed. Comprehensive knowledge about the manifestations and evaluation of CC among primary care providers and emergency room physicians is essential to prevent delays in diagnosis and treatment.

Table 1 Characteristics of Patients with CC (N=40)

Demographics
Age: Mean 42.8y with SD 14.08 Sex: Male 24 (60%), Female 16 (40%) Ethnicity: Hispanic 23 (57.5%), non-Hispanic 17(42.5%) Race: Caucasian 28 (70%), African American 2 (5%), Asian 6(15%), Others 4 (10%)
Comorbidities and Risk factors
Diabetes mellitus: 6 (15%)

Smoking: 9 (22.5%) Immunosuppressed due to medications: 3(7.5%) Alcohol abuse: 6 (15%) Substance abuse: 5 (12.5%) Outdoor occupational exposure: 14 (35%)
Pathology (Biopsy of the skin lesion)
Biopsy was available 16 (40%) Granulomas 10 (25%) Necrosis 4 (10%) Inflammation 9 (22.5%) Spherules 4 (10%) Others 1 (2.5%) Multiple findings 9 (22.5%)
Initial diagnosis
Cocci (22.5%) Erythema nodosum (15%) Bacterial cellulitis (37.5%) Tinea (7.5%) Cysts 2 (5%) Multiple abscesses 2 (5%) Mass 1 (2.5%) Nodules 1 (2.5%) Lumps 1 (2.5%) Blister/Bullae 1 (2.5%)

55 POSTER

CONTINUED EXPLORATION OF THE STATISTICAL LINKS BETWEEN COCCIDIOIDOMYCOSIS CASES AND WILDLAND FIRES

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Abstract

INTRODUCTION: Wildland fires are a known source of dust and bioaerosol emissions and a documented cause for a localized coccidioidomycosis outbreak among wildland firefighters. We hypothesize wildland fires may also be a source for transporting Coccidioides and increases in coccidioidomycosis case counts may be attributable to wildland fire activity.

METHODS: We explored statistical relationships between wildfire activity and changes in coccidioidomycosis case counts in California from 2000-2019. We collected fire perimeter data from the CAL FIRE Wildfire Perimeters and Prescribes burns dataset, as well as wildfire emission and smoke data from the Global Fire Emissions Database (GFED; Version 4.1) and the EPA's Community Multiscale Air Quality Modeling System (CMAQ). We gathered coccidioidomycosis case data from the California State Department of Health.

RESULTS: We are continuing to explore the statistical relationships between coccidioidomycosis incidence/cases and emissions. So far, we have found no statistical relationship between coccidioidomycosis cases and wildfire activity through GFED. We are working on a causal analysis.

CONCLUSION: Determining whether wildland fires are a cause for long-range transport of *Coccidioides*, or a cause for increased coccidioidomycosis cases in the surrounding region due to some other circumstance, will benefit mitigation efforts and messaging surrounding coccidioidomycosis risk.

56 POSTER

CHARACTERIZATION OF IMMUNE RESPONSES TO COCCIDIOIDES INFECTION IN PIGTAIL MACAQUES

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Abstract

INTRODUCTION: The development of a *Coccidioides* vaccine is imperative as the endemic regions of the pathogen spread due to climate change. Animal models that closely model Valley Fever infection in humans are needed to gain a better understanding of the types of immune responses a vaccine will need to induce, and to evaluate candidate vaccines for immunogenicity and efficacy. Nonhuman primates are the closest model to humans in their anatomy, physiology, immune responses, and susceptibility to infections. Pigtail macaques bred at the Washington National Primate Research Center (WaNPRC) in Mesa, AZ are susceptible to *Coccidioides*, and their range of clinical manifestations closely align with human symptoms. Here, we characterized immune responses in pigtail macaques naturally exposed to *Coccidioides*, with the goal of gaining a better understanding of the immune responses responding to the infection.

METHODS: Four female pigtail macaques (4-19 years, 5.55-9.70 kg) at the WaNPRC were sampled for blood and bronchoalveolar lavage (BAL). All animals had either a history of *Coccidioides* infection, or active *Coccidioides* infection. Cryopreserved samples from four pigtails with no known history of *Coccidioides* infection were used as controls. Immune cell phenotypes in whole blood and bronchoalveolar lavage (BAL) were characterized by flow cytometry to measure the frequency and activation status of innate and adaptive immune cell subsets. Cryopreserved PBMC were also assessed for T cell responses by ELISpot and intracellular cytokine staining (ICS). PBMC were stimulated with overlapping peptide pools representing *Coccidioides* expression library immunization antigen 1 (ELI_Ag1), antigen 2/proline-rich antigen (Ag2/PRA), and peroxisomal matrix protein1 (PMP1). IL-17 and IFN-γ responses to *Coccidioides* antigens were measured using an Immunospot Human IFN-γ/IL-17 Double-Color ELISPOT Kit. In addition, the frequency of CD4+ and CD8+ T cells expressing IL-17, IL-22, IL-4, IFN-γ, TNFα, IL-2, and Granzyme B/CD107a (marker of cytolytic effector function) were measured by ICS.

RESULTS: Animals with a history of *Coccidioides* infection exhibited higher levels of activated CD4+ and CD8+ T cells in the BAL. In addition, IFN-γ responses to all 3 peptides measured by ELISPOT as well as CD8+ T cell responses (collectively IFN-γ, TNFα, IL-2, Granzyme B and CD107a) measured by ICS were elevated in *Coccidioides*-exposed NHP with the strongest responses in an animal that more recently tested positive. The strongest T cell responses were detected against PMP1.

CONCLUSION: These results show that pigtail macaques naturally infected *Coccidioides* develop elevated immune activation in the lung. In addition, natural infection results in a broad repertoire of T cell responses to several immunogens. Limitations of this work include highly variable immune responses among the four exposed animals that was likely due to variable sampling timepoints relative to first positive test for VF and may not have captured peak *Coccidioides* immune responses. These findings support development of a preclinical nonhuman primate natural and experimental infection models for the evaluation of new vaccines and therapies for the prevention and treatment of Valley Fever.

58 ORAL

SMALL MAMMAL BURROWS SHAPE THE DISTRIBUTION OF *COCCIDIOIDES* IN SOILS: EVIDENCE FROM A LONG-TERM ECOLOGICAL EXPERIMENT IN THE CARRIZO PLAIN NATIONAL MONUMENT, CALIFORNIA, USA

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Abstract

Introduction. The importance of zoonotic involvement in the lifecycle of *Coccidioides* spp., the soil-dwelling fungal pathogen that causes coccidioidomycosis, is debated. Evidence that rodents are hosts for *Coccidioides* spp., combined with the fungus' ability to degrade animal keratin, has given rise to the hypothesis that asymptomatic rodent hosts serve as zoonotic reservoirs, releasing Coccidioides spherules into the environment upon death. While researchers have widely established a higher probability of *Coccidioides* spp. detection in rodent burrows compared to surface soils, prior studies have been unable to disentangle the effect of rodent occupation of burrows from the effect of the burrow microhabitat.

Methods. The Carrizo Plain National Monument is California's largest intact grassland, and coccidioidomycosis is known to be endemic to the region. In 2007, a long-term experimental study was initiated in the Carrizo Plain whereby rodents were excluded from certain areas (20 by 20 meters each) via the installation of children wire fences dug 1 meter below ground. Within the exclosures (n=20), the original burrows were left intact, permitting disentanglement of the effects of burrowing hosts on *Coccidioides* spp. from the effects of burrows. Over four sampling periods spanning April 2021 to April 2022, we collected 1,861 soil samples using a factorial design that crossed burrows and topsoils (i.e., top 10 cm of soil) with exclosures and non-exclosures. We collected information on soil moisture, temperature, and vegetation, and analyzed soils for presence of *C. immitis* DNA. We conducted a causal mediation analysis with g-computation to identify the extent to which extent burrowing mammals influence the distribution of *C. immitis*, either directly or indirectly via modification of the soil environment (e.g., burrow creation).

Results. *C. immitis* DNA was detected in 28.3% (219/774) of the burrow samples taken from outside rodent exclosures, and 18.7% (72/387) of the burrow samples collected from within the exclosures. Only 3.3% (13/389) of the topsoil samples outside rodent exclosures, and 1.0% (4/385) of the topsoil samples taken from within the exclosures, had detectable *C. immitis* DNA. In generalized linear mixed models that controlled for soil conditions, rodent presence, and sample type (burrow vs. surface soil), we estimated that the odds of detecting *C. immitis* when rodents are present is 2.5 (95% CI: 1.6, 3.4) times higher than when rodents are absent, and that the odds of detecting *C. immitis* is 17.4 (95% CI: 16.9, 18.0) times higher when soils are taken from burrows compared to top soil. Lower soil moisture was the only abiotic factor associated with greater odds of C. immitis detection (OR: 0.86, 95% CI: 0.75, 0.97). In mediation analyses, we estimated that rodent presence increases the odds of *C. immitis* detection by a factor of 26.4 (95% CI: 16.6, 44.6), and that 70.6% (66.1, 75.7%) of this total effect is mediated via burrows.

Conclusions. Our results indicate that burrowing hosts play a critical role in shaping the distribution of *C. immitis* in soils, with the majority of the effect attributable to burrow creation. While our results suggest that burrows may be highly suitable microhabitats for *C. immitis* even in the absence of host occupation, rodent exclusion was associated with significantly lower odds of *C. immitis* detection. Fifteen years after rodents were excluded from certain areas, the probability of *C. immitis* detection in burrows with limited rodent access was 34% lower than in burrows where rodents had continuous access.

59 ORAL

COCCIDIOIDOMYCOSIS: KNOWLEDGE, ATTITUDES, AND PRACTICES AMONG HEALTHCARE PROVIDERS – ARIZONA, 2014

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Introduction

In 2014, a coccidioidomycosis specific Knowledge, Attitudes, and Practices (KAP) survey was implemented to evaluate the diagnosis and treatment behaviors of Arizona providers. With interest in reporting differences from respondents of varying clinical degree and by receipt of continued medical education (CME).

Methods

Similar to the 2007 KAP analysis, the survey consisted of 42 Likert-scale, dichotomous, or multiple-choice questions. Questions were based on 2005 clinical practice recommendations from the Infectious Disease Society of America (IDSA) and from suggestions provided by subject-matter experts. Eligible participants, as determined by 2013 records from the Arizona Board of Nursing and Arizona Medical Board, consisted of practicing and licensed nurse practitioners (3,205) and medical and osteopathic physicians (8,819) in the state of Arizona. Data was processed and assessed using SAS® Version 9.4. Continuous variables were evaluated for normality using the Kolmogorov-Smirnov Test, while Mid-P exact values were estimated by chi-square tests for association of attributes; level of significance at the alpha = .05 level. Confounded by age, as determined by the 10% rule, multivariate logistic regression models were employed to evaluate the association between clinician behaviors and respondent clinical degree or CME.

Results

Of 1,054 returned surveys, only 841 (628 physicians and 213 nurse practitioners) were included in the final analysis (7.1% response rate) as records missing a clinical degree or those completed by a physician's assistant were excluded. Almost all practitioners (99.5%, 812/816) expressed that coccidioidomycosis is a problem in Arizona, with 98.8% (684/692) of respondents agreeing that disease incidence has risen in the past ten years. Provider confidence in ability to diagnose coccidioidomycosis (83.2%, 584/702) was reported at higher frequencies, as compared to confidence in treating the fungal condition (72.8%, 496/681).

Relative to nurse practitioners, physicians were less likely to report counseling patients when ordering a diagnostic test (OR = 0.53, 0.32-0.88; p=.014) and test patients who presented with community-acquired pneumonia (CAP) for coccidioidomycosis (OR=0.55, 0.38-0.78; p=.001). Yet, only 56.4% (97/172) of nurse practitioners and 44.3% (243/549) of physicians reported testing for coccidioidomycosis when a patient presents with CAP. Nurse practitioners were more likely to report offering treatment to newly diagnosed patients, regardless of clinical scenario (e.g., asymptomatic with positive serology, with bilateral pneumonia, etc.).

Of all respondents, 16.3% (137/841) received coccidioidomycosis related CME within the past 3 years, with 69% of nurse practitioners and 45% (284/628) of physicians reporting clinical education in Arizona. Relative to providers without CME, those who received CME within the past 3 years were more likely to counsel patients when ordering a diagnostic test (OR=2.64, 1.41-4.95; p=.002) and when diagnosed with coccidioidomycosis (OR=3.73, 1.33-10.46; p=.012). In addition, those with CME were far more likely to test patients who present with CAP as compared to their counterparts (OR=2.55, 1.69-3.85; P=>.001).

Conclusion

While those surveyed demonstrated comprehensive knowledge of coccidioidomycosis, nurse practitioners and physicians remained susceptible to errors in their practice; straying away from 2005 IDSA clinical recommendations. With only 16% reporting Coccidioidomycosis related CME within the past 3 years, it suggests the need to further promote and target specific clinical fields with educational opportunities.

60 ORAL

THERAPEUTIC DRUG MONITORING OF ANTIFUNGAL AGENTS IN PATIENTS WITH COCCIDIOIDOMYCOSIS

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Abstract

Introduction: Coccidioidomycosis, is a fungal infection that can vary widely in presentation and severity, often requiring chronic treatment with triazole antifungals. Therapeutic drug monitoring (TDM) has historically been implemented to optimize use of drugs with narrow therapeutic indices and has not been common practice for most azole antifungals. Given the complexity of assessing treatment response in coccidioidomycosis, which can be impacted by medication adherence, pharmacokinetics, host immune response, and fungal response, TDM of azole antifungal therapy has been proposed to improve clinical evaluation of such variables. Consistent TDM is anticipated to ultimately increase the probability of a successful outcome in the treatment of coccidioidomycosis.

Methods: This is a retrospective review of patients with coccidioidomycosis who received adjunctive antifungal TDM at Kern Medical from May 2011 to December 2022. Approval and a waiver of consent were obtained from the Institutional Review Board. The combination of a patient and azole antifungal was considered a unique therapy regimen, with each azole course evaluated separately for patients who received multiple azoles over the course of their coccidioidomycosis treatment. Data extracted from electronic health records included demographics, baseline comorbidities, clinical presentation and dissemination, routine Coccidioides serologies, cerebrospinal fluid analysis, antifungal medication treatment (dosage, frequency, and start date), and outcomes.

Results: We reviewed the electronic records of 1015 patients with coccidioidomycosis and azole drug levels. Fluconazole comprised the majority of these courses (89%), with the remainder divided between itraconazole, posaconazole, voriconazole, and isavuconazole. Of the serum azole levels obtained, 32% were within the therapeutic range, 66% were subtherapeutic, and 2% were supratherapeutic.

Conclusion: Therapeutic drug monitoring is a useful tool that advantages the care of patients with coccidioidomycosis. This tool assists clinicians in better ascertaining when patients were nonadherent, or in case of under or overdosing on their azoles. This would assist in preventing misdiagnosis as a failure of therapy and potential side effects.

61 ORAL

DNA AND SELF-AMPLIFYING RNA VACCINES EXPRESSING *COCCIDIOIDES* ANTIGENS INDUCE ROBUST MUCOSAL AND SYSTEMIC IFN-γ AND TH17 T CELL RESPONSES IN MICE

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INTRODUCTION: Studies in mice and humans show IFN-γ and Th17 T cell responses are associated with control of *Coccidioides* infections indicating that an effective vaccine will likely need to induce these responses. In addition, we reasoned that localization of these responses in the lung mucosa may provide better protection at the site of exposure. Nucleic acid vaccines, including both DNA and RNA vaccines, induce robust CD4+ and CD8+ T cell responses including IFN-γ and Th-17 responses. Here, we investigated the feasibility of using our novel DNA and self-amplifying RNA vaccine platforms to induce IFN-γ and Th17 T cell responses to known *Coccidioides i*mmunogens in mice and the effects of co-delivering genetic adjuvants designed to increase systemic and mucosal T cell responses.

METHODS: The feasibility of self-amplifying replicon RNA (repRNA) and DNA vaccines expressing the *Coccidioides* immunogens expression library immunization antigen 1 (ELI_Ag1), antigen 2/proline-rich antigen (Ag2/PRA), and peroxisomal matrix protein1 (PMP1) for the ability to induce mucosal and systemic IL-17 and IFN-γ responses in mice. The repRNA vaccine was delivered at 1 or 10mg doses by either a novel Lipid InOrganic Nanoparticle (LION) (HDT Bio) or by gene gun delivery (1mg dose) directly into epidermal cells in the skin. The DNA vaccines (1mg dose) were also delivered by gene gun but since DNA vaccines are not inherently self-adjuvanting, they were co-administered with two plasmids expressing the following genetic adjuvants: 1) A mucosal adjuvant, heat-labile enterotoxin from E. coli (LT) and 2) a Th1 stimulating adjuvant, IL-12. Mice were primed and boosted 4 weeks apart. Three weeks after the booster dose, splenocytes and lung lymphocytes were collected from each mouse, stimulated with peptide pools representing each vaccine immunogen and then IL-17 and IFN-γ responses were measured using an Immunospot Mouse IFN-γ/IL-17 Double-Color ELISPOT Kit.

RESULTS: Both the repRNA and adjuvanted DNA vaccines administered by either LION or gene gun induced robust (200-1700 SFC per 106 lymphocytes) systemic and mucosal IFN-γ and/or Th17 responses to PRA/AG2 and PMP1 in the spleen and lung but only the DNA vaccine induced significant T cell responses to ELI_Ag1 in both the spleen and lung. Overall, the adjuvanted DNA vaccine induced the strongest T cell responses to all 3 immunogens in both the spleen and the lung. All vaccines induced both IFN-γ and Th17 responses, but the Th17 responses were strikingly stronger in the lung mucosa.

CONCLUSION: RNA and DNA vaccines have historically been employed primarily for viral vaccines. These results establish our RNA and DNA vaccine platforms as potent strategies to induce robust IFN-γ and Th17 T cell responses against *Coccidioides*. In addition, our results suggest that co-delivery of strong mucosal and Th1 adjuvants could modulate and increase induction of both systemic and mucosal immune responses that may improve protection. Studies in progress will compare antibody responses induced by these vaccine platforms and the determine protective efficacy of these vaccines from *Coccidioides* challenge in mice.

62 ORAL

CRANIOCERVICAL COCCIDIODOMYCOSIS MENINGITIS: MANAGEMENT IMPLICATIONS FOR HYDROCEPHALUS, SYRINGOMYELIA AND CNS DRUG DELIVERY

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Abstract

BACKGROUND: Craniocervical structural anomalies causing obstructive hydrocephalus and syringomyelia are well known in the neurosurgical population. Progressive craniocervical coccidioidal meningitis with associated arachnoiditis and intradural inflammatory masses may mimic these conditions. Exacerbation of pre-existing hydrocephalus and development of syringomyelia can occur from mechanical obstruction and alteration of CSF flow dynamics. Complete obstruction of ventricular CSF outflow due to arachnoiditis may further limit CNS penetration and delivery of antifungal agents into the subarachnoid space. Early recognition and neurosurgical treatment of craniocervical arachnoiditis may lead to clinical improvement of its associated manifestations.

CASE DESCRIPTION: A 25-year-old male with a 2-year history of disseminated coccidioidomycoses meningitis and shunted hydrocephalus was emergently admitted for acute worsening of hydrocephalus presumably due to a VP shunt malfunction. He previously had failed fluconazole therapy and had been on voriconazole with therapeutic drug levels He was noted to have developed low pressure hydrocephalus requiring VP shunt removal followed by several days of external ventricular drainage for CSF diversion. A diagnostic workup revealed an enhancing intradural dorsal mass at the posterior craniocervical junction causing CSF obstruction. Serial imaging revealed the rapid development of a high cervical cord syringomyelia. This latter finding in addition to what now appeared to be obstructive hydrocephalus complicating communicating hydrocephalus prompted surgical decompression. Additionally, an intraventricular dye study revealed complete obstruction of ventricular CSF outflow due to arachnoiditis. Thus, mechanical blockage of oral or intravenous antifungal agents in reaching the CNS meninges would not be unexpected and negatively affect medical therapy. The patient underwent a suboccipital craniectomy, C1 laminectomy, duraplasty followed by continued external ventricular drainage. Once the patient's brain compliance improved with improvement of hydrocephalus, he underwent placement of a new VP shunt.

CONCLUSIONS: VP "shunt failure" in shunted coccidioidal meningitis patients may not necessarily indicate a shunt malfunction per se. Development of worsening hydrocephalus may also occur due to poor brain compliance and low-pressure hydrocephalus and craniocervical intradural masses/arachnoiditis causing obstructive hydrocephalus. The appearance of a syringomyelia involving the high cervical cord should raise suspicion and prompt further diagnostic investigations. Early recognition and surgical intervention are essential to minimize progressive neurological deficits.

63 POSTER

UTILIZING NATURALLY OCCURRING SOIL MICROBES IN ARIZONA TO INHIBIT THE GROWTH OF COCCIDIOIDES SPP

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Abstract

Introduction: While little is known about the potential for microbial competition in soil, preliminary observations suggest that several soil bacteria have the capability to inhibit the growth of *Coccidioides* by releasing metabolites. *Bacillus pumilus* and *Bacillus subtilis* produce metabolites that have been shown to inhibit and slow the growth of C. posadasii when co-cultured together. Analyzing the metabolites released from the co-cultured competing soil microbes will contribute to a deeper understanding of what the specific chemical composition is. Microbes identified as antagonists of *Coccidioides*, and/or the metabolites they secrete, have the potential to be used as natural biocontrol agents to limit the fungal burden at geographic point sources, ultimately reducing the potential for human infection.

Methods: In-vitro challenge assays were utilized to characterize the antagonistic, synergistic, and neutral relationships of native soil microbes against *C. posadasii*. The microbes were isolated from known *Coccidioides* positive soil sites in Tucson, AZ. Secreted metabolites of microbes co-cultured on agar plates with *C. posadasii* were extracted from the zone of inhibition using a standard methanol extraction. The agar in the zone of inhibition was removed using a sterile scalpel and briefly frozen at -80oC for 24 hours in a 50 mL vented conical to inhibit growth. 20 mL of methanol was added to the conical and shaken at 30 rpm at room temperature for 24 hours. The methanol was allowed to evaporate before 5 mL of sterile deionized water was added to the conical and lyophilized. High Performance Liquid Chromatography (HPLC) was used to screen for any crude metabolites extracted from agar to narrow down range prior to analysis via Matrix-Assisted Laser Desorption/Ionization Mass Spectroscopy (MALDI-MS). Mass-to-charge (m/z) ratios were calculated and used to compare ratios of reported crude compounds using a known database.

Results: When co-cultured with *Coccidioides* spp., two species in the bacterial genus *Bacillus* significantly inhibited the radial growth of *Coccidioides*. Bacterial competitors, *Bacillus pumilus* and *Bacillus subtilis* were shown to decrease radial growth. HPLC was used to screen respective samples for the presence of any compounds. HPLC showed that there were peaks at different timepoints, indicating that there are different compounds associated with each organism. MALDI-MS input m/z ratios were compared to known compound m/z ratios using the UCSD Metabolomics Workbench [1]. Significance of metabolites were determined by relevance to microbial synergist/antagonist capabilities found in literature. We were able to narrow down 19 likely compounds from this study, some of which have cytotoxic and antifungal properties.

Conclusion: We have found that several species in the *Bacillus* genus inhibit growth of *Coccidioides* in-vitro, particularly *B. pumilus* and *B. subtilis*. These findings indicate that Bacilli bacteria can be potential candidates for biocontrol agents. The most likely metabolites secreted from these organisms and knowledge gained from these initial experiments will be used to optimize the isolation and identification process in future studies.

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64 POSTER

FATAL COCCIDIOIDOMYCOSIS CASES IN AN ENDEMIC AREA

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Abstract

Introduction. While a majority of Coccidioides infections are asymptomatic, it is estimated that 1% develop severe disease and can be fatal. Prior retrospective reviews that attempt to identify risk factors for the fatal disease have been limited to public health data hampered by reporting challenges and accuracies. Morbidity review of fatal cases identified by clinical criteria rather than ICD-9/ICD-10 reporting may improve the accuracy of conclusions. The aim of this novel study at the conclusion is to describe a comprehensive case series of patients with fatal coccidioidomycosis.

Method. At an academic center in an endemic area, medical records from three sequential electronic medical record systems were reviewed between the years 2000 to 2023. Patients with coccidioidomycosis were identified by microbiological, pathological, or serological testing criteria. Deaths were determined by in-hospital records, insurance reporting, review of death certificates if available, or public records. Demographics, clinical course, outcomes, and causes of death are compared.

Results. To date, 25 patients met the clinical criteria for having coccidioidomycosis at the time of death. ICD coding has been inaccurate in about half of the cases during the screening phase. Data integrity has been a challenge when extracting data across three electronic medical record systems. Comorbidities included diabetes mellitus type II, hypertension, cirrhosis, HIV/AIDS, and COVID-19. Certain patients were treated with amphotericin B at the time of death. When amphotericin was used it was administered intrathecally or intravenously. Medication adherence was variable prior to death. CF Titers ranged from <1:2 to >1:512. Time from diagnosis to death ranged from days to a decade. A patient may have one or more sites of dissemination including the osseous, integumentary, central nervous system, liver, lymph nodes, and adrenal glands. Certain disseminated CNS cases exhibited hydrocephalus that required a shunt and CSF analysis with WBC > 1200, Glucose <30, and protein greater than 3,000.

Causes of death included cardiac arrest, acute respiratory failure, renal failure, dysautonomia and septic shock.

Conclusion. Patients were found to have died either from coccidioidomycosis or with coccidioidomycosis. ICD coding was inconsistent. A precise and accurate description of coccidioidomycosis staging is important when trying to determine risk factors for a fatal disease.

65 POSTER

USING MOLECULAR METHODS TO EXAMINE THE PRESENCE OF COCCIDIOIDES IN WILD MAMMALS IN ARIZONA AND THEIR LUNG FUNGAL COMMUNITIES

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Introduction. The presence of *Coccidioides* spp. in the soil has previously been shown to be associated with rodent burrows (Kollath et al., 2020). However, the role of small mammals in the life cycle of the fungus remains unclear. One current hypothesis is that small mammals are environmental reservoirs for *Coccidioides* which has been supported by recent studies (Sharpton et al., 2019; Taylor and Barker, 2019). Therefore, this study aims to assess the prevalence of Coccidioides in mammal lungs in Arizona and the lung fungal communities associated with the presence or absence of Coccidioides.

Methods. Lung samples from wild kangaroo rat (n=4), pocket mouse (n=7), pack rat (n=2), grasshopper mouse (n=2), antelope-ground squirrel (n=3), round-tailed ground squirrel (n=1), desert cottontail (n=6), antelope jackrabbit (n=2), black-tailed jackrabbit (n=2), and mule deer (n=1) were obtained from the Arizona Game and Fish Department. Lung samples were stored at -20C until DNA extraction using the Qiagen Blood and Tissue Kit (Qiagen, Valencia, CA). Samples were screened (1) for the presence of Coccidioides using the CocciDx real-time qPCR assay to detect a Coccidioides-specific region present throughout the genome of both *C. immitis* and *C. posadasii*, and (2) for fungal communities through ITS2 Illumina amplicon sequencing. Data was analyzed to obtain the relative abundance of the fungal orders within the lung communities, the Bray-Curtis measure of dissimilarity (Beta-diversity), and the Shannon diversity index (Alpha-diversity).

Results. Three pocket mice and one antelope-ground squirrel tested positive for Coccidioides; all other species tested negative. Both antelope-ground squirrel and pocket mice displayed higher abundance of Onygenales fungi compared to the other mammal species in this study. The fungal communities found in the lungs of pocket mice had a lower alpha-diversity than in the other mammal species tested. Lung fungal communities of pocket mice and antelope-ground squirrel were similar between themselves and different than the communities found in other species.

Conclusion. Fungal communities in the lungs of the species that tested positive for Coccidioides were similar between themselves and different from the communities in the lungs of the species that tested negative for Coccidioides.

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66 ORAL

IDENTIFICATION OF A NOVEL VIRULENCE FACTOR IN *COCCIDIOIDES POSADASII*

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Abstract

Introduction: Although the genus *Coccidioides* is divided into two closely related and putatively allopatric species analysis shows that hybridization has occurred between species, and at least one *C. posadasii* conserved fragment has introgressed in a population-specific manner. Transcript abundance *in vitro* and *in vivo* for ten ORFs in this introgressed region were measured for several isolates. We used signals of introgression and high mRNA transcript levels in the spherule as strong indicators of selection for genes related to critical biological processes involved in *Coccidioides* pathogenesis. The only transcript in the introgression region with significant expression was a gene that encodes for a beta-defensin-like (DEFBL) peptide rich in serines and cysteines. Few virulence factors have been identified in *Coccidioides*, and we employed the CRISPR-Cas9 mediated gene deletion tool to delete this gene in *Coccidioides*.

Methods: To elucidate function, we first used a bioinformatics approach to determine homology to other known proteins and characterize various conserved features. We then deleted the DEFBL gene (CPSG05265) in *C. posadasii* strain Silveira. Initial attempts to delete the gene using homologous recombination and Agrobacterium-mediated transformation were unsuccessful, possibly a result of the introgression dynamics at the locus. We used an *in vitro* assembled double cut CRISPR-Cas9 methodology to delete then target, which we adapted from Aspergillus fumigatus. Briefly, we designed 50-bp of microhomology to the 5' and 3' regions flanking the gene immediately adjacent to PAM (NGG) sites targeted by the guide RNA, and using hi-fidelity PCR created a hygromycin resistance cassette to replace the DEFBL locus.

We measured radial growth on solid agar media and spherule and endospore size and number. Growth curves from daily growth measurements for all conditions were compared using linear regression analysis in R. To assess virulence, a murine model of coccidioidomycosis was used. C57BL/6J mice were used for survival and CFU *in vivo* for *Coccidioides*. Ten mice per group (6 to 8-weeks old) were infected intranasally by insufflation with 100 conidia of Silveira or deletion strain. Mice were sacrificed at 30 days or when moribund. Lungs were extracted and processed for CFU; spleens and brains cultured *in toto* to determine dissemination. To assess pathogenesis, 6- to 8-week-old C57BL/6J were given an experimental respiratory infection as described above. Mice were sacrificed in a time series, lungs were extracted and ½ processed for histopathology, ½ processed for CFU. CFU from spleen and brain was assessed to determine if a reduction in dissemination observed in the preliminary results is replicated, and analysis of circulating cytokines with ProcartaPlex cytokine/chemokine panels were completed.

Results: Analysis of transcript differences between the two life phases of *Coccidioides* spp. revealed the abundance of the transcript was 24-fold higher in the parasitic phase than the saprobic phase. Our initial investigation of the expression of DEFBL *in vivo* indicates it is also present during infection. However, the function of the gene is elusive, with greatest homology to a beta-defensin-like peptide in *Anole carolinensis* and toxins in spider venom. Only one minor homologous hit in a fungal organism was found in the entomopathogenic bio-control agent *Metarhizium anisopliae*. Analysis using SignalP suggests the protein is cleaved and secreted. Classic beta-defensins in vertebrates are characterized by six cysteine residues in the form C-X6-C-X4-C-X9-C-X6-C-C. The sequence motif in both species of *Coccidioides* is C-X6-C-X5-C-X7-C-X5-C-X-C; thus, the molecule is termed a beta-defensin-like peptide. DEFBL genes commonly encode toxic peptides of reptiles and anti-microbial peptides in various organisms. Small, secreted peptides typically consist of a signal peptide, a pro-segment sequence followed by a ~30AA defensin with the six cysteine residues that create 3 disulfide bridges and are 2-6kD. Sequence analysis confirms that DEFBL in both species of *Coccidioides* contain all these features. Protein alignment indicated that there are 3 amino acids deleted in the *C. immitis* sequence

type, and an arginine to leucine change at position 71. Interestingly, the *C. posadasii* sequence type of the gene is found in *C. immitis* strain RS, which is one of the common virulent lab strains 4.

We created a gene deletion in *C. posadasii* using the double-cut CRISPR-Cas9 technology with success. We have confirmed deletion of DEFPL-cp using PCR and Southern blot. Loss of function mutants grow normally on solid media but produce smaller spherules. The mutant also causes less severe disease, with lower fungal burden and dampened immune response, most notably a reduction in IL-1beta and TNF-alpha.

Conclusion: This novel protein, identified using genomic analyses, appears to be a virulence factor. Due to a lack of homology with other fungal proteins, its exact function remains elusive. Ongoing work will seek to determine localization and interaction with other proteins. Identification of novel virulence factors is necessary to increase our knowledge of mechanisms of pathogenesis in *Coccidioides*.

67 POSTER

EXPLORING THE FUNCTION OF *COCCIDIOIDES* GENES THROUGH CRISPR/CAS9

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Abstract

Introduction:
Coccidioides provides a unique challenge when identifying genetic functions and, to date, there is a limited amount of information about individual genes within the *Coccidioides* genome. This renders the need for unique approaches in identifying, characterizing, and understanding the role and impact of various genes associated with the *Coccidioides* life cycle. The clustered regularly interspaced short palindromic repeats (CRISPR) using a CRISPR-associated protein 9 (Cas9) enzyme system provides a targeted approach to independently remove individual genes of interest (GOI) while simultaneously leaving the rest of the genome intact, and ideally, unchanged. During removal, the GOI is replaced with an antimicrobial resistance selection marker that, when grown on the associated selection media, will select for isolated colonies of *Coccidioides* where the GOI has been removed. Utilization of the CRISPR/Cas9 approach provides the ability to independently study and better understand the role that the GOI plays within Coccidioides's various functions and interactions with other organisms and the environment (e.g., growth, pathogenicity, spherule production, etc.).

Methods:
GOIs are chosen via various methods prior to designing associated guide RNAs (gRNAs) and replacement sequences. Linear Constructs are ~1600bp replacement sequence gBlocks ordered from Integrated DNA Technologies (IDT) containing the hygromycin resistance gene with an additional 100bp of homology sequence on either end that correlates with the 5' and 3' sequences flanking the GOI. To isolate transformable cells, *C. posadasii* strain Silveira arthroconidia are incubated overnight until they reach the germling life stage. Following methods developed for *Aspergillus fumigatus*[1], germlings are subjected to enzymatic digestion to remove all cell wall and debris, leaving transformable protoplasts. The genomic DNA is targeted for editing via an adapted double cut CRISPR Cas9 enzyme system[1] to delete the GOI before being replaced with the gBlock gene replacement by homologous recombination. Transformed protoplasts are plated onto GYES agar plates and incubated for 48hr at 30°C. After 48hr growth, a GYE soft agar containing Hyg B is overlaid onto the growth plates and then again incubated at 30°C for an additional 4-7 days. Once growth is confirmed, individual colonies are isolated onto new plates containing 2xGYE with HYG that are incubated for 7 days at 30°C. All putative transformants are additionally passaged on 2xGYE with HYG for 7 days at 30°C until phenotypically pure before DNA extraction to screen for successful transformants. DNA is subjected to marker-specific PCR and a GOI-specific PCR to confirm complete transformation. Confirmed transformants are moved forward for further characterization.

Results:
We have used CRISPR/Cas9 editing to effectively transform *Coccidioides*, which supports future work in understanding of the mechanisms behind pathogenicity and additional functions. These transformed isolates have been used to study various mechanisms including, but not limited to, pH sensing and spherule production.

Conclusion: CRISPR/Cas-9 transformation of *Coccidioides* provides the ability for a better understanding of the role of each GOI in relation to *Coccidioides*'s various functions. As such, this method supports development of various treatments, identification of pathogenicity and virulence factors, and expands the understanding of the molecular mechanisms of dimorphism of *Coccidioides*.

1 Al Abdallah, Q., Ge, W. & Fortwendel, J. R. A Simple and Universal System for Gene Manipulation in Aspergillus fumigatus: In Vitro-Assembled Cas9-Guide RNA Ribonucleoproteins Coupled with Microhomology Repair Templates. mSphere 2 (2017). https://doi.org:10.1128/mSphere.00446-17

68 ORAL

STRUCTURAL AND FUNCTIONAL ANALYSIS OF CPS1; A VIRULENCE FACTOR IN VALLEY FEVER

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Abstract

INTRODUCTION: An avirulent *Coccidioides posadasii* strain has shown promise as a vaccine candidate against Valley Fever infections in canines and humans. Here we examine the deleted gene, *CPS1*, to determine a mechanism of action using structural and functional methods.

METHODS: To determine the enzymatic function and structure of the orphan Cps1 protein we utilized a recombinant DNA plasmid, and a galactose inducible S*accharomyces cerevisiae* expression system. Cells were lysed by bead beating and poly-his tagged Cps1 resided in the crude membrane fraction of the cellular lysate. The recombinantly expressed protein was purified via nickel affinity chromatography and size exclusion chromatography. A multitude of techniques including biochemical assays, cryo-electron microscopy (cryoEM) and Alphafold2.1 structure prediction aided in deorphanizing Cps1 activity.

We examined the biochemical mechanism of the potential fatty acid ligase using substrates predicted from sequence and structural homology. Adenosine triphosphate (ATP) binding was probed with a fluorescent ATP analog and the result was supported by a protein thermal shift in the presence of ATP. Further enzymatic characterization was performed with a colorimetric ATP hydrolysis assay in the presence of purified protein, fatty acid, ATP, and coenzyme A. Confocal fluorescence microscopy was used on *S. cerevisiae* cells expressing Cps1-GFP and counter stained against either mitochondria with MitoTraker™ Red or vacuolar membrane with FM4-64.

We generated an Alphafold2.1 structural prediction which yielded a protein model for further analysis. The protein surface charges were calculated in ChimeraX and suggested the protein is peripherally associated to the membrane and not an integral membrane protein. The predicted model was also queried in the DALI server and a high throughput structural alignment against experimental structures revealed similar protein folds and function. Purified protein was subjected to cryoEM structural elucidation on a 300 kV Titan Krios electron microscope. Sample was applied to copper quantifoil grids and plunge froze in liquid ethane before the collection of a 641-micrograph dataset. Data analysis was performed in RELION4.

RESULTS: Cps1 is a hydrophobic protein with a link to virulence in *C. posadasii*. Our results show that the protein is peripherally associated with membranes within the cell, interacting through charged lipid head groups and charged residues on the protein surface. Cps1 resides in the crude membrane fraction of cell lysate and is extracted from the membranes with 1.0 M NaCl. Confocal microscopy reveals Cps1 colocalizes with mitochondrial membranes although it is expected to act at membrane contacts between mitochondria and the vacuole. Purified Cps1 binds to ATP-TNP, a fluorescent ATP analog, and ATP-TNP can be displaced using unlabeled ATP yielding a binding affinity of 4.1 mM. Alphafold2.1 modeling and subsequent structural alignments suggest Cps1 shares the greatest structural and sequence similarity to acyl-CoA and acyl-AMP ligases.

Using a molybdate based ATP hydrolysis assay, we showed that ATP hydrolysis is most stimulated in the presence of oleic acid, ATP, and coenzyme A, suggesting the product formation of oleoyl-CoA. CryoEM structural analysis reveals a low-resolution initial model of a highly symmetrical Cps1 dimer. The experimental dimer has the same dimensions of two alphafold predicted monomers and in vitro formaldehyde crosslinking shows a highly stoichiometric dimer formation on SDS-PAGE; however, the functional consequence of this dimer is not known.

CONCLUSIONS: *CPS1* gene deletion is accompanied by a loss of virulence in *Coccidioides*, however, there is no annotated function for Cps1. Here we show Cps1 enzymatic activity, membrane association, subcellular localization, and oligomeric state of Cps1 that suggest Cps1 functions as an oligomeric enzyme in fatty acid modification localized to mitochondria.

69 POSTER

DISSEMINATED COCCIDIOIDOMYCOSIS PRESENTING AS AN OVARIAN MASS

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Abstract

Introduction: Coccidioidomycosis, colloquially known as Valley fever, is an endemic fungal infection predominantly localized to the San Joaquin Valley. While a more common presentation is a community-acquired pneumonia, it can also disseminate systemically to other organs and body cavities, including the peritoneum. We put forward a case of disseminated coccidioidomycosis in a young African American female presenting as an ovarian mass.

Methods: Retrospective case report

Results: 29-year-old African American female with mild intermittent asthma presented with symptomatic abdominal distension for one month. One year prior, she had suffered gunshot wounds to scalp, left shoulder and left hand. Trauma survey found no internal injuries but did incidentally find a 7mm noncalcified pulmonary nodule. She underwent left shoulder surgical repair one year later. Post operatively she had persistent throat pain thought to be due to intubation. She was given steroids and morphine. Eventually the pain resolved over three weeks. One month after the surgery, she noted abdominal distension. This progressed until she developed dyspnea when lying flat for which she presented to outside hospital where she was found to have right adnexal mass, ascites, and elevated CA-125. Due to concern for gynecological malignancy, the patient underwent diagnostic laparoscopy, exploratory laparotomy, right salpingo-oophorectomy, and omentectomy. Pathology found diffuse necrotizing granulomatous inflammation with spherules with endospores consistent with *Coccidioides* species without evidence of malignancy. Intraoperative tissue culture grew Coccidioides immitis with fluconazole MIC 8 (UT San Antonio). She had positive immunodiffusion IgM and IgG serology with complement fixation titers of 1:128. Nuclear medicine bone scan found no evidence of bony metastasis. She was started on fluconazole 800mg daily with durable contraception in place.

Conclusion: Our case highlights the importance of including coccidioidomycosis in the differential diagnosis for female patients with incidental pulmonary nodules that present with gynecological and constitutional symptoms masquerading as a cancerous process after a steroid exposure, especially in a region endemic to Valley fever.

70 ORAL

PROTEIN MICROARRAYS IDENTIFY KNOWN AND NOVEL IMMUNOREACTIVE *COCCIDIOIDES* PROTEINS

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Abstract

Introduction We created a protein microarray of 605 *Coccidioides* proteins based on proteins previously reported using mass-spectrometry of spherule-phase extracts. We probed the arrays with sera from dogs with Community-Acquired Coccidioidomycosis (CAC (N 10) and non-immune controls (N=7) as well as serum samples taken 2, 4, 6, and 8 weeks after laboratory infections (Laboratory-Acquired Coccidioidomycosis, LAC, N=53). The goal of this study was to identify novel sero-reactive proteins.

Methods The protein array was created using a process called the Nucleic Acid Programmable Protein Array (NAPPA) technology. Briefly, coding regions of the proteins were synthesized in an expression vector so that the Coccidioides protein would have a C-terminal fusion with Glutathione-S-Transferase (GST). The plasmids were printed individually into nanowells of a silicon-etched microscope slide along with an anti-GST antibody. On the day of the experiment, the proteins were produced by a coupled in-vitro-transcription/translation reaction, and the nascently produced proteins captured by the anti-GST antibody and displayed for immunoreactivity studies. Canine serum samples were used to probe the arrays. We utilized fluorescently-labeled secondary antibody to detect anti-canine IgG. The fluorescent intensity of the binding was measured with a microarray scanner. The data were normalized to the median intensity of the array. A median-normalized value of > 1.5 fluorescent units was used as a cutoff value to calculate percent seropositivity. We utilized sero-reactivity of 20% or greater within a given population to select potential hits for further validation.

Results One of the prominent proteins identified was the known serologically important protein, Complement Fixation (CF) antigen, also known as CTS-1. As an initial quality control, we tested the array performance with sera from CAC dogs which were also known to be positive in immunodiffusion assays to CF (CF-Positive). Canine serum samples that were non-reactive (CF-Negative) in immunodiffusion assays were also run on NAPPA. Some controls on the array included fungal extracts Coccidioidin Lysate (CDN-L) and Coccidioidin Filtrate (CDN-F) antigenic preparations. Reactivity to NAPPA-CF protein by the CAC canine sera showed 100% specificity and 100% sensitivity. This was expected given that the sera were positive by CF immunodiffusion prescreening. Sera from the LAC canines were run blinded to the NAPPA team. The results were decoded after performing the analysis. The pre-infection LAC canine sera were non-reactive to CF. At week 2 post-infection, one canine showed anti-CF reactivity. At week 4, eight out of ten LAC canines produced anti-CF antibodies. In weeks 6-8 100% of the LAC canines were immunoreactive to CF, as well as other coccidioidal proteins. We identified the top 19 reactive of the 605 Coccidioides proteins in both the CAC and LAC cohorts with a criterion of >20% positivity and an odds ratio of >2. The CAC canines reacted to 36 proteins while the LAC canines reacted to 55 proteins on the array. Cross-referencing these two lists, we identified 20 proteins that were reactive in both CAC and LAC canines. In this protein list, two proteins were in the chitinase family. We also identified two other reported immunoreactive proteins, peroxisomal matrix protein and 4-hydroxyphenylpyruvate dioxygenase. This leaves us with 16 novel proteins that were not previously known to be sero-reactive.

Conclusions We present the results of a study of infected canines and their response to a 605-Coccidioides protein member array. The differences between the CAC and LAC immune responses are not unexpected. We hypothesize that the CAC canines generally have a lower-dose challenge and are exposed to genetic variants as opposed to a high dose of a single isolate in the LAC cohort. The results reveal sero-reactive proteins in dogs that were previously unknown to elicit antibody responses. Future experiments will include analyzing the reactivity of serum samples from dogs infected with other fungi and other infectious agents. We hypothesize that combining multiple antigens in an immunodiagnostic assay will improve the current quality of immunodiagnostics.

71 ORAL

LUNG EPITHELIAL CELLS REGULATE THE IMMUNE RESPONSE TO INTRANASAL VACCINATION AGAINST COCCIDIOIDOMYCOSIS

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Abstract

Introduction: *Coccidioides* spp. are inhaled primary pulmonary pathogens. Lung epithelial cells act as a physical and immunological barrier against inhaled pathogens but have not been investigated in regulating resistance to infection. A live attenuated vaccine has been shown to protect mice against experimental infection after being given subcutaneously or intranasally. We investigated the role of lung epithelium in mice vaccinated mucosally.

Methods: We studied mucosal vaccination using a live attenuated strain of C. posadasii lacking CPS1 (Δcps1), which has been shown to protect C57BL/6J mice against a lethal infection with wild type (WT) Coccidioides spp. Mice received 10e5 spores intratracheally as described for vaccine induced resistance. We studied the role of the lung epithelium in mobilizing early inflammatory responses by using conditional transgenic mice in which epithelial subsets could be eliminated during vaccination.

Results: C57BL/6J mice that received intranasal Δcps1 spores accumulate neutrophils, other myeloid cells, innate lymphocytes, and T and B cells within their lungs by 24hrs, with responses increasing by 72hrs. This inflammation is sharply attenuated in conditional mice that are depleted of club cells just prior to vaccination. Club cell-deficient mice show reduced accumulation of neutrophils in their lungs 24hrs after vaccine delivery, with additional impairments in the accumulation of monocytes, DCs, γδ T cells, and B cells by 72 hrs, when compared to non-depleted controls. We studied how club cells coordinate the vaccine response and discovered that the lectin-mediated complement pathway plays a prominent role. Δcps1 spores are sensed via mannose-biding lectin 2 (MBL2) and ficolin-1 (FCN1), which prompt rapid accumulation of the anaphylatoxin C3a in the lung airway. C3a binds to the C3a receptor (C3aR), a G-protein coupled receptor (GPCR) on the club cells, activating downstream signaling and early inflammatory responses. Blockade of C3aR with a selective antagonist or inhibition of Gi-GPCR adenyl cyclase signaling in club cells in vivo in conditional DREADD mice retards inflammation in the lungs of vaccinated mice.

Conclusions: Our work illustrates the unappreciated, requisite the role of lung epithelium in assembling immunity in response to intranasal vaccination against coccidioidomycosis. We would expect that the lung epithelium may likewise exert a significant role in the early host response to wild-type spores and initial control of coccidioidomycosis.

ORAL PRESENTATION ABSTRACTS

COCCIDIOIDOMYCOSIS CLUSTER AMONG WILDLAND FIREFIGHTERS, CALIFORNIA 2021

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INTRODUCTION: In July 2021, the California Department of Forestry and Fire protection (CAL FIRE) notified the California Department of Public Health (CDPH) of seven wildland firefighters from the same fire crew who developed respiratory symptoms concerning for coccidioidomycosis. CDPH and CAL FIRE began an investigation to identify and confirm cases and better understand coccidioidomycosis risk among these wildland firefighters.

METHODS: CDPH reviewed medical records and conducted standardized phone interviews with the three firefighters with confirmed coccidioidomycosis.

RESULTS: Illness onset dates and work history suggested exposure to Coccidioides likely occurred while working on a fire in late June 2021 in the California Central Valley. All interviewed patients reported heavy dust exposure, two disclosed having heard of Valley fever before being tested for it, none reported wearing respiratory protection, and all were hospitalized. CAL FIRE was proactive about recommending coccidioidomycosis testing following possible exposure, and cases were diagnosed within 12 days as compared to a median of 55 days from onset to diagnosis reported generally for coccidioidomycosis.

CONCLUSION: As coccidioidomycosis and wildfire frequency increase in California, additional exposure prevention tools, increased awareness, and early recognition and disease management are needed to protect wildland firefighters.

CONTRIBUTION OF BIOLOGIC RESPONSE MODIFIERS TO THE RISK OF COCCIDIOIDOMYCOSIS SEVERITY

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INTRODUCTION: The risk of coccidioidomycosis (CM) as a life-threatening respiratory illness or disseminated CM (DCM) increases as much as 150-fold in immunosuppressed patients. The safety of biologic response modifiers (BRMs) as treatment for patients with autoimmune disease (AI) in CM-endemic regions is not well defined. We sought to determine that risk in the Tucson and Phoenix areas.

METHODS: We conducted a retrospective study reviewing demographics, Arizona residency length, clinical presentations, specific AI diagnoses, CM test results, and BRM treatments in electronic medical records of patients ≥18 years old with International Classification of Diseases (ICD-10) codes for CM and AI from 1 October 2017 to 31 December 2019.

RESULTS: We reviewed 944 charts with overlapping ICD-10 codes for CM and AI, of which 138 were confirmed to have both diagnoses. Male sex was associated with more CM ($P = .003$), and patients with African ancestry were 3 times more likely than those with European ancestry to develop DCM ($P < .001$). Comparing CM+/AI+ ($n = 138$) with CM+/AI– ($n = 449$) patients, there were no significant differences in CM clinical presentations. Patients receiving BRMs had 2.4 times more DCM compared to pulmonary CM (PCM).

CONCLUSIONS: AI does not increase the risk of any specific CM clinical presentation, and BRM treatment of most AI patients does not lead to severe CM. However, BRMs significantly increase the risk of DCM, and prospective studies are needed to identify the immunogenetic subset that permits BRM-associated DCM.

HEALTH DISPARITIES IN COCCIDIOIDOMYCOSIS INCIDENCE — CALIFORNIA, 2000–2019

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Background: Coccidioidomycosis is a fungal infection caused by soil-dwelling Coccidioides immitis or posadasii, which people can contract by inhaling spores from the environment. Although most infected persons recover without symptoms or with mild illness, ~1% of patients develop severe disseminated disease that can result in death. In California, incidence rates are highest among persons aged 40–59 years and people who self-identify as Black or Hispanic or Latino. We sought to estimate association between coccidioidomycosis incidence rates and California's Healthy Places Index (HPI), a metric of community health, to guide public health practice and messaging toward less advantaged populations in California.

Methods: We analyzed California coccidioidomycosis cases reported during 2000–2019. Patients' residential addresses were geocoded, linked to data from the 2010 census, and categorized into 4 HPI quartiles based on census tract HPI score. Tracts in the lowest scoring HPI quartile (HPI 1) are less advantaged as measured by 25 health equity variables. For each HPI quartile, we calculated age-adjusted incidence by sex, age, and race. Multivariable negative binomial regression was used to calculate incident rate ratios and assess trends.

Results: In total, 74,622 coccidioidomycosis cases were reported in California during 2000–2019; incidence rate/100,000 population was highest among people aged 40–59 years (11/100,000 population), males (10.6/100,000 population), and persons aged 20–39 years (9.3/100,000 population). Overall, incidence was highest in HPI 1 and decreased with increasing HPI scores (incidence rate ratio HPI 1 vs HPI 4 = 7.03, P <0.001). When stratifying by HPI, the highest incidence rate was for persons aged 40–59 years in HPI 1 (21.7/100,000 population).

Conclusions: In California, coccidioidomycosis incidence rates were highest in less advantaged communities. Socially and culturally appropriate guidance for coccidioidomycosis outreach programs, testing, and treatment should be strengthened for these communities.

WORK-RELATED COCCIDIOIDOMYCOSIS IN CALIFORNIA: EXAMINING THE BURDEN OF DISEASE USING WORKERS' COMPENSATION AND ARCHIVAL DATA

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INTRODUCTION: Coccidioidomycosis is a health hazard for workers in endemic areas; however, systematic analysis of work-related disease has been challenging due to limitations in available data.

METHODS: I examine the potential burden of work-related coccidioidomycosis using two data sources. First, I analyze 2240 claims submitted to California's Workers Compensation Information System (WCIS) for coccidioidomycosis from 2000 to 2019 using R Studio. This work was supported by a collaboration with the California Department of Public Health – Occupational Health Branch and the California Department of Industrial Relations – Division of Workers Compensation. I obtained IRB approval for working with confidential WCIS data. I calculated the number of claims by employers' industry, worker occupation, sex, and age at time of injury. I produced preliminary disease incidence rates for 5-year periods using data from the American Community Survey. WCIS data can only capture disease among workers who submitted a claim to workers' compensation. To complement the analysis, I systematically collected data from state agency investigations and reports, news media, and legal cases and built an archival database of over 100 work-related exposures and outbreaks in California.

RESULTS: The WCIS data and the archival data point to similar findings. First, in line with broader disease surveillance, reports of work-related disease have increased. Incidence rates from the WCIS data have doubled from .39 out of 100,000 workers between 2000-2004 to a high of .83 between 2015-2019. Second, the top three Census industries reporting work-related disease include Public Administration (44%), Construction (14%), and Agriculture (9%). Third, the most common Census occupations included Protective Service Occupations (28%), Construction and Extraction Occupations (18%), and Healthcare Practitioners and Technical Occupations (9%). Fourth, most work-related disease is reported by men (82%). However, reports from women (65%) outnumber claims from men in healthcare occupations and analysis of injury descriptions suggests that 73% of laboratory-based exposures occurred among women.

CONCLUSION: This research provides both an up-to-date and systematic analysis of work-related coccidioidomycosis in California and suggests future directions for work-related disease prevention efforts.

REACTIVATION OF COCCIDIOIDAL DISEASE PROGRESSION IN ASYMPTOMATIC, STABLY INFECTED MICE

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Introduction: Reactivation of coccidioidomycosis in humans is reported under conditions of severe immunosuppression, such as AIDS and solid organ transplantation. In a new model of chronic, stable infection in mice, dexamethasone suppression was applied in a pilot study of the loss of infection control.

Methods: B6D2F1 mice were infected IN with 50 spores of the less virulent *C. posadasii* strain 1038 (Cp1038) and rested for 5 weeks. On day 37 p.i., dexamethasone (DXM) was added to the drinking water at a rate of 6 mg/L. Control mice received plain water. Mice (2 per time point and treatment) were sacrificed on days 0, 5, 10, 15 and 20 for histopathology (left lung) and flow cytometry (right lung). For flow cytometry, granulomas in the right lung were dissected closely with a 3 mm skin biopsy punch and placed in RPMI. Left lungs were fixed in 4% paraformaldehyde for 24 hrs and moved to 70% ethanol. Multiparameter flow cytometry assessed total cell counts in granulomas, viability, and myeloid and lymphoid cells and subsets. Five micrometer sections of the left lung were stained routinely with H&E.

Results: Histopathology of baseline and untreated mice revealed well-organized granulomas with a necrotic center, a fibrogranulomatous mantle region, and lymphoid aggregates on the borders of the mantle. Spherules were estimated at 0-1 per 400X field and were all located within the borders of the necrotic center. By day 5, neutrophil populations were diminished in mice receiving DXM and there was an increase in spherules as well as observation of them outside the previous borders of the necrotic center. Lymphoid aggregates were still observed. By day 15, there was a decrease in all immune cell types and the lesions ceased to have any organization. Lymphoid aggregates were not present, replaced by sheets of plasma cells. On day 20, large numbers of neutrophils had returned to the mixed inflammatory lesion, fibrosis was not observed, and spherule numbers were >20 per 400X field, dispersed throughout the lesion. Flow cytometry revealed that by day 5 in treated mice, there was a 1-1.5 log reduction in total cells and in T cells, B cells, and neutrophils. Neutrophils rebounded robustly by day 20 while T-cell numbers remained depressed.

Conclusion: This observational study demonstrates that there are rapid changes in spherule numbers and cell populations in the previously controlled granulomas when immunosuppressive doses of DXM are administered to mice. This shows that reactivation can be modeled in mice and suggests a plethora of cellular and molecular studies to understand the host pathogen relationship in controlled and reactivated coccidioidomycosis.

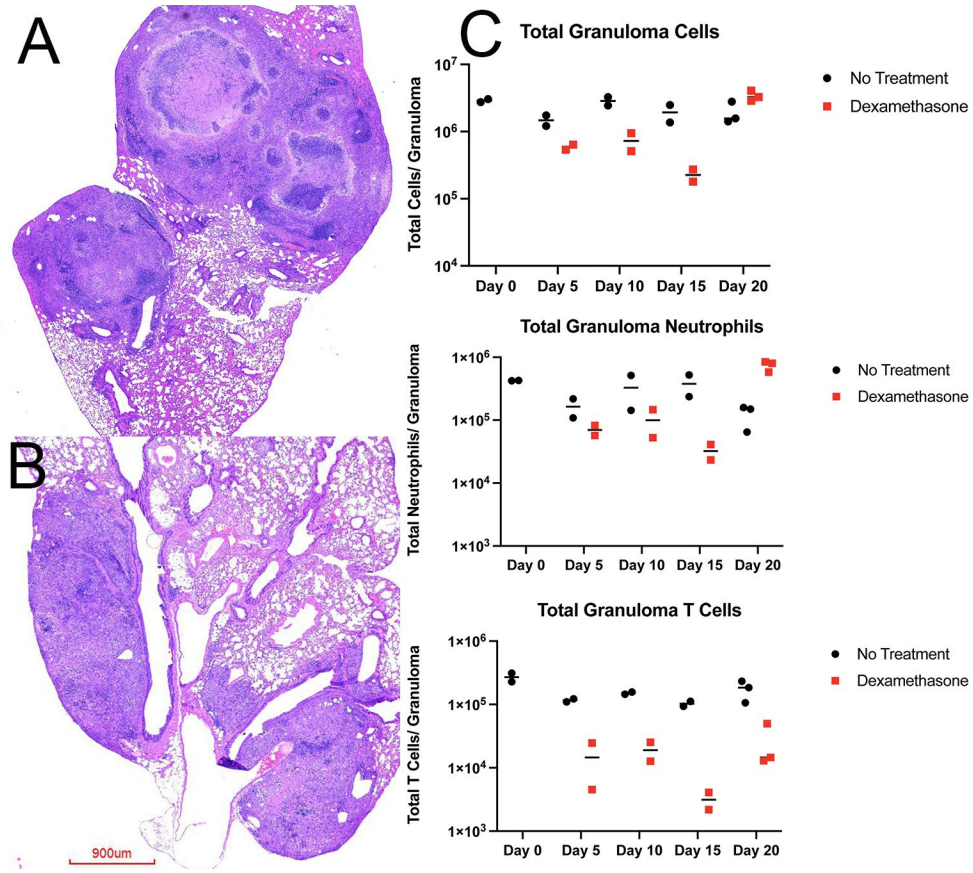

Figure: A) Normal granulomas in untreated B6D2F1 mouse. Necrotic center contains neutrophils and spherules with a thick fibrogranulomatous mantle and lymphoid aggregates on the borders of the mantle. **B)** B6D2F1 mouse after 20 days DXM administration. There are few lymphocytes, and the neutrophils have recovered but there is total loss of granuloma structure; **C)** Total cell numbers in the granulomas decrease >1 log by day 15 and rebound by day 20. T-cell populations reduce and do not recover; the cell increase is due to neutrophils. (A and B, H&E stain, magnification 1.3x and 1.4x, respectively; C, Vicell - granuloma viable cell counts, flow cytometry - T-cell ($CD3^+$) and neutrophil ($CD11B^+$, $Gr\text{-}1^+$) counts)

COCCIDIOIDOMYCOSIS SCORE SYSTEM: MSG 2.0

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Introduction: In 1981 the Mycoses Study Group (MSG) developed a scoring system to quantify severity of fungal infections. The score system was modified for coccidioidal infection treatment trials with azole drugs. The Valley Fever Institute has endeavored to develop a score system specifically for coccidioidomycosis that is less subjective than the original. The intent was to develop a score system that was more specifically applicable to the great variety of coccidioidal illness: pulmonary, disseminated, meningeal. It is important to measure the patients' perception of health to assess benefit of health care interventions. We included the PRO/QOL as a choice of questionnaires to establish patient perceptions of changes in their health and satisfaction.

Methods: We evaluated the original scoring system and multiple studies that used this in the evaluation of Coccidioidomycosis. Variables that are difficult to measure are eliminated. Variables that were easily reproduced were added.

Results: Included in the revised non-meningeal scoring system are weight loss, eosinophilia, markers of inflammation, skin tests, imaging, and coccidioidal serology. There is a separate section for pulmonary disease with newly included physiologic scoring for severity. Specific sections for skin, subcutaneous abscess, joints, bone, intraabdominal, lymph nodes, and other new disseminated sites are included.

The revisions to the MSG scoring system for meningeal disease are simplified and objectified. The evaluation of mental status is modernized. A new section for increased intracranial pressure is added to include this critical advance in knowledge about initial and subsequent patient care.

Conclusions: The goal of this effort is to update the 1981 MSG scoring system by incorporating newer understanding of coccidioidal disease into the numerical standardized format. We are endeavoring to improve usability, reliability and pertinence of outcome measurement of any anti coccidioidal therapy. Future validation studies are planned. The addition of validated patient related outcomes measures adds a new dimension to evaluating therapeutic interventions.

AN OUTBREAK OF COCCIDIOIDOMYCOSIS AMONG GEOLOGY STUDENTS

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Introduction: The fungal arthroconidia Coccidioides species are known to be endemic in the soil of multiple areas in the Southwest dessert United States, most commonly San Joaquin Valley of California. In October of 2021, a group of geology graduate students traveled to multiple locations in the Carrizo Plains located at southeastern San Luis Obispo County to map and study the local geology. The group was unaware of the potential for substantial exposure to Coccidioides in the area evaluated. No specific remediation was taken to prevent the accusation of airborne arthroconidia. It is interesting to note, this outbreak engrafted during the peak time of the SARS-CoV-2 pandemic. The purpose of this report is to extend knowledge of the epidemiology of Coccidioides in southwest California.

Methods: This study was approved by the Caltech Institutional Review Board. A retrospective chart review was performed on all 12 patients using electronic health records. A literature search was conducted on PubMed and google scholar using the following search terms: valley fever, coccidioidomycosis.

Results: In Fall 2021, a group of 12 Caltech individuals traveled to multiple locations in the Carrizo Plains to map and study the local geology. The first identified case occurred when a student was admitted to the hospital for progressive respiratory symptoms and a rash. A diagnosis of coccidioidomycosis (CM) was made by a consulting infectious disease specialist. A second student was also hospitalized around the same time for “nonspecific” viral symptoms which was also eventuated in the diagnosis of pulmonary CM. A third student presented to Caltech student wellness (CSW) services for prolonged respiratory symptoms with the onset of three weeks upon returning from the trip. One week following the trip the professor also developed progressive protracted respiratory symptoms and was seen at an outside facility and diagnosed with CM. This pattern of exposure was identified by the physician at CSW who then coordinated evaluation, diagnosis, and care for all 12 members of the group. Definitive immunoassay tests were performed by the UC Davis Coccidioidomycosis Serology Laboratory, resulting in a final confirmation of 5/12 positives, 4 of whom required treatment.

Conclusions: There are many unknown areas adjacent to San Joaquin Valley of California that are endemic to Coccidioides such as Southeastern San Luis Obispo County. This report should serve as a warning to individuals that participate in outdoor activities such as hikers, campers, geologists and construction workers to be wary of the potential risk of exposure to Coccidioides. The clinicians also should be aware of newly found endemic areas and have a low threshold to test for identification of Coccidioides.

IMMUNOGENETICS ASSOCIATED WITH SEVERE COCCIDIOIDOMYCOSIS

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Introduction: Disseminated coccidioidomycosis (DCM) is caused by Coccidioides, pathogenic fungi endemic to Western United States and Mexico. Illness occurs in approximately 30% of those infected, <1% of whom develop disseminated disease.

Methods: We enrolled DCM patients, performed whole-exome sequencing and assessed cytokine production in stimulated peripheral blood mononuclear cells (PBMC). Confocal microscopy co-localized DECTIN-1 and fungal endospores. Transfection demonstrated DECTIN-1, PLCG2, DUOX1 and DUOXA1 roles in b-glucan-stimulated H2O2 production. RNA was sequenced from STAT3-mutated, autosomal-dominant Hyper-IgE syndrome patients (AD-HIES) respiratory tissues. Duox1-/- mice were infected with Coccidioides.

Results: In an exploratory set of 67 DCM patients, two had haploinsufficient STAT3 mutations. Defects in b-glucan sensing and response were seen in 34/67 (50.7%) cases. Damaging CLEC7A (n=14) and PLCG2 (n=11) variants were found and PBMC from patients with these variants produced less b-glucan-stimulated TNF-a than healthy controls ($P<0.005$). Using ancestry matched controls, damaging variants in CLEC7A and PLCG2 were over-represented in DCM ($P=0.0206$, $P=0.015$, respectively) including CLEC7A Y238* ($P=0.0105$) and PLCG2 R268W ($P=0.0025$). In a validation cohort of 112 DCM patients PLCG2 R268W ($P=0.0276$), CLEC7A I223S ($P=0.044$), and CLEC7A Y238* ($P=0.0656$) were confirmed. Fifteen discovery cohort patients had heterozygous DUOX1 or DUOXA1 variants which impaired H2O2 production in transfected cells. AD-HIES patient airway epithelial cells had decreased DUOX1 and DUOXA1 transcripts. Duox1-/- mice had increased morbidity and mortality following Coccidioides infection.

Conclusions: Patients with DCM have impaired b-glucan sensing or response affecting H2O2 production. Genetically impaired Coccidioides recognition and cellular response decrease inflammatory cytokine production and are associated with disseminated coccidioidomycosis.

INTEGRATING PUBLIC HEALTH SURVEILLANCE AND ENVIRONMENTAL DATA TO MODEL THE PRESENCE OF COCCIDIOIDOMYCOSIS.

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INTRODUCTION: Due to regional differences in public health surveillance and under detection of infections, it is challenging to use reported coccidioidomycosis case data to characterize true disease risk. However, statistical modeling methods can help fill in this information, including in areas that do not have mandated reporting.

METHODS: Using monthly, county-level coccidioidomycosis case data and various environmental and socioeconomic characteristics, we use a binary model that estimates the unobserved presence of *Coccidioides*, while accounting for imperfect detection of coccidioidomycosis cases in Arizona, California, Nevada, New Mexico, and Utah from 2000-2015.

RESULTS: We estimate the presence of *Coccidioides* was associated with higher temperatures and soil moisture levels. Using these statistical relationships, we are able to map the county-level estimates of coccidioidomycosis case burden across the southwestern United States, providing a better understanding of the endemic areas and the presence of Coccidioides.

CONCLUSION: This work aims to help inform future surveillance needs and clinical awareness for coccidioidomycosis. Additionally, this approach can estimate the probability of presence of coccidioidomycosis in places that don't report.

COCCIDIOIDES DETECTED IN RODENT BURROW SOILS, BUT UNDETECTED IN AGRICULTURAL SOILS OR SETTLED DUST, IN THE SAN JOAQUIN VALLEY IN CALIFORNIA

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Introduction: The reported incidence of coccidioidomycosis in the United States has increased by approximately six hundred percent over the past twenty years. Much is known about the geographic distribution of coccidioidomycosis cases, though comparatively little is known about the causative organism, *Coccidioides*, as to how it exists in the natural environment. A better understanding of the environmental biology of *Coccidioides*, particularly its seasonal dynamics, would aid in disease prevention and mitigation strategies.

Methods: Here, nearly one thousand soil and settled dust samples from the San Joaquin Valley in California were collected over the course of multiple years, and the presence of *Coccidioides* was determined using the CocciEnv qPCR assay. Additionally, the composition of the fungal community was determined using ITS metabarcoding. Approximately half of the samples were from four agricultural sites (surface soils, soil cores and settled dust) spanning 160km from Bakersfield, California to just south of Fresno, California. The remaining samples were from five undeveloped sites (rodent burrow soils and settled dust) on an 80km north-south transect along California highway 33.

Results: *Coccidioides* was detected in approximately one third of rodent burrow soil samples, with positive samples collected from all five undeveloped sites. *Coccidioides* was not positively detected in any soil samples from the four agricultural sites investigated and was undetected in all settled dust samples regardless of site. Where *Coccidioides* was positively detected, detection was strongly associated with the site where samples were collected, though unassociated with the month of collection. Detection of *Coccidioides* was not strongly correlated with the β-diversity (community structure) of the greater fungal community in the soils sampled. The two undeveloped sites with the highest *Coccidioides* detection rate were adjacent to substantial washes, whereas the remaining 3 undeveloped sites were not.

Conclusion: There is a high likelihood of finding *Coccidioides* in rodent burrows along California highway 33. It is possible that rodent burrows are the primary source of *Coccidioides* spores in the area around California highway 33, as the *Coccidioides* detection rate here far exceeds that of soils in general. *Coccidioides* was not positively detected in soils from the four agricultural sites studied here, suggesting that it is unlikely that *Coccidioides* would be found in soils on other agricultural fields in the San Joaquin Valley. The strong differences in the probability of detecting *Coccidioides* between individual highway 33 sites, and the lack of an association between *Coccidioides* detection and sampling month, indicate that spatial variables may be more important for predicting *Coccidioides* presence than temporal variables. It is not clear why *Coccidioides* was undetected in settled dust, especially at undeveloped sites along highway 33 where *Coccidioides* is prevalent in rodent burrow soils.

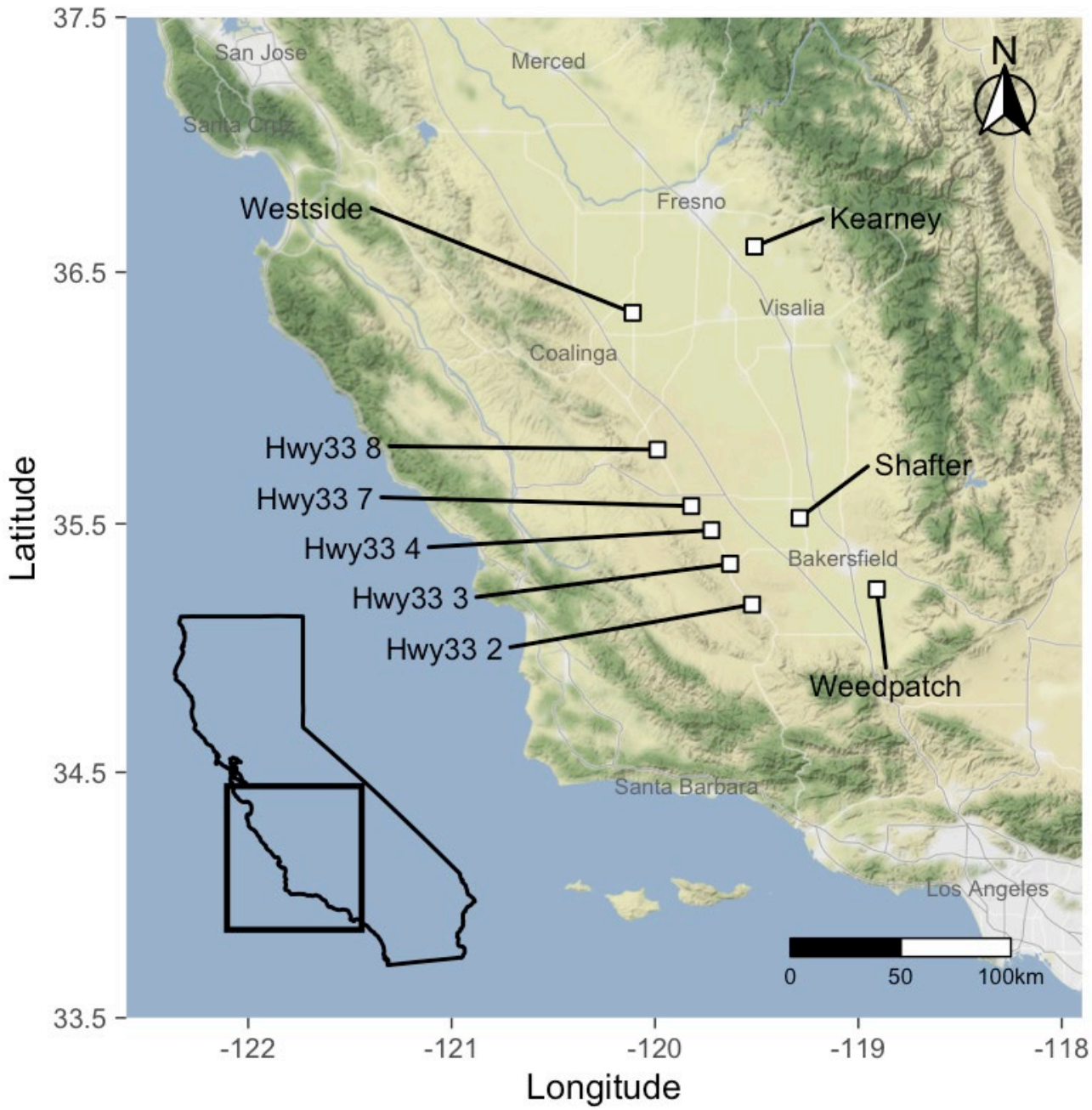

Figure 1: Map of soil collection locations

RISK FACTORS FOR FLUCONAZOLE FAILURE IN THE TREATMENT OF COCCIDIOIDAL MENINGITIS

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INTRODUCTION: Azole therapy is the current standard of treatment for coccidioidal meningitis (CM). Guidelines from the Infectious Disease Society of America (IDSA) recommend oral fluconazole at a dose of 400 to 1200 mg daily as initial therapy. However, many patients fail therapy with fluconazole and require alternate agents for treatment. Robust data regarding treatment are lacking. We aim to understand risk factors for fluconazole failure.

METHODS: We conducted a single-center retrospective chart review of patients from our institution with CM, and identified patients using an electronic search of International Classification of Disease (ICD) versions 9 and 10, using codes 114.2, B38.4, and B38.9. We included patients with biochemical evidence of meningitis and positive serology, antigen, polymerase chain reaction or culture in the cerebrospinal fluid (CSF). We excluded patients without adequate treatment details, suspected but not laboratory-confirmed coccidioidal meningitis, and those who were not initiated on fluconazole as the first line of treatment. We defined fluconazole failure as any sustained increase in meningitis-related symptoms or progression of CSF or imaging abnormalities, with resultant medication change (either increase in fluconazole dosage or change to another medication for reasons other than fluconazole adverse effects or drug toxicity). This study was approved by the Mayo Clinic Institutional Review Board. Descriptive statistics were used for data analysis. Chi squared goodness of fit test was used for categorical variables and Analysis of Variance (ANOVA) was used for continuous variables.

RESULTS: From 1/1/99 to 5/15/21 we identified 102 patients with CM, and excluded 31 based on exclusion criteria, yielding 71 patients studied. Among the 71, 22 (31%) experienced fluconazole failure, requiring either increased dosage of fluconazole or alternate antifungal treatment. No statistically significant predictors of failure were found amongst the demographics, clinical characteristics, laboratory indices, and imaging findings between patients who failed fluconazole and those who did not. Patients who were previously treated for non-CNS coccidioidomycosis (N=19) had a higher rate of fluconazole failure (40.9% vs 20.4%), though the difference was not statistically significant ($p = 0.07$). Initial dosage of fluconazole was not found to be a statistically significant predictor of fluconazole failure (400 mg failure rate 7/17[41.2%] versus 800 mg failure rate 13/44[29.5%], $p = 0.39$). The median time to fluconazole failure was 7.7 months (range 2.0-25.5); the median time to failure was longer for patients with a prior diagnosis of non-CNS coccidioidomycosis (22.8 vs 6.5 months, $p=0.25$). There was insufficient data to compare time to failure by fluconazole dosing.

CONCLUSION: One-third of CM patients at our institution who were initiated on therapy with fluconazole failed treatment. Our study did not find any statistically significant predictors of failure, including initial dosage of fluconazole. A larger study may have detected a small difference. A randomized control trial would be ideal to further evaluate CM outcomes and ascertain risk factors for failure and most appropriate fluconazole dose for therapy.

QUANTITATIVE BIOMARKERS TO DETERMINE RISK OF DISSEMINATED COCCIDIOIDOMYCOSIS

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Introduction: Coccidioidomycosis is probably the endemic mycosis of most clinical importance. Multiple modalities have been established as diagnostic. This includes culture and histopathology. More recently, antigen testing and polymerase chain reaction have found limited applicability. The mainstay of diagnostics for almost 100 years has been serology for IgG and IgM antibodies. There are multiple modalities for measuring these antibodies. There is major variation between the results of this test in terms of sensitivity and specificity. Most of these tests lack sensitivity in early disease and do not define successful treatment with clarity. Therefore raison d'être for new test technology. This report explores a pilot study of coccidioidal RNA measurement as in early and more sensitive diagnostic.

Methods: Kern Medical has developed a biobank of specimens from the great diversity of patients with Coccidioidomycosis. The biobank was approved by the Kern Medical IRB. Thereby, patients were consented for use of their biologic specimens. 12 patients were selected from the biobank, 5 with disseminated disease and 7 with disease clinically limited to the lung at time of specimen collection. Circulating RNA was extracted from serum samples with a KingFisher (ThermoFisher), circulating RNA was profiled with RealSeq® proprietary technology. This technology takes advantage of novel developments to detect circulating RNAs from host and pathogens with high accuracy. Samples were sequenced with Illumina sequencers and data was analyzed using RealSeq's proprietary RiboMarker® bioinformatics pipeline.

Results: All disseminated cases had measurable coccidioidal specific RNA, two patients with primary disease had measurable coccidioidal specific RNA, and five patients with primary disease had undetectable coccidioidal specific RNA.

Conclusion: Coccidioidal RNA analysis shows promise as a diagnostic test with broad applicability. It is possible that it could serve as an early diagnostic test with increased sensitivity, and it conceivably could be developed to the holy grail of cocci testing - a test of control cure.

MODELING THE DISCHARGE OF INFECTIOUS ARTHROCONIDIA OF THE FUNGAL PATHOGEN *COCCIDIOIDES POSADASII* GROWING IN SOIL

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INTRODUCTION: The inhalation of airborne infectious arthroconidia from the fungal pathogen *Coccidioidies posadasii* initiates the disease coccidioidomycosis (Valley fever). Being the only method of infection, the process of conidia discharge is essential to understanding the disease dynamics of Valley fever. Our aim was to quantify the amount of arthroconidia being discharged from soil over time and to study the effects that moisture and precipitation have on this biological process

METHODS: Soil was inoculated with the pathogen and grown under varying temperature and moisture conditions. Arthroconidia was trapped on a filter and quantified weekly with a hemocytometer. We fit a logistic growth curve model to estimate and predict discharge of each condition through time. This model is used to test hypotheses of peak temporal discharge of conidia.

RESULTS: The analysis shows that under all environmental conditions there is a logistic discharge pattern of arthroconidia that peak 4 weeks after inoculation followed by a plateau. Low moisture leads to a significant decrease in conidia production, although a high abundance is still produced under low moisture conditions. Arthroconidia are consistently being discharged into the ambient air despite no significant soil disruption.

CONCLUSION: The purpose of this study was to temporally quantify the production of infectious arthroconidia and to predict conditions that stimulate an increase in abundance in the environment that can lead to a surge in infections. A better understanding of this process allows for more robust disease surveillance and improved knowledge of the biology and ecology of this organism.

EXPLORING CYCLES OF COCCIDIOIDOMYCOSIS USING PATTERNS OF RELATIVE INCIDENCE IN ENDEMIC COUNTIES IN THE UNITED STATES

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Introduction: Coccidioidomycosis incidence varies substantially by time and place within the western United States, and the contributing factors, including public health surveillance biases like changes in diagnostics and environmental influences, are poorly understood. It is especially challenging to disentangle spatiotemporal changes in incidence caused by environmental factors, such as weather or climate, from those caused by concurrent differences in surveillance across time and space. We used national surveillance data to explore cycles of coccidioidomycosis incidence at the county level, focusing on relative rather than absolute patterns in incidence to control for the effects of surveillance biases.

Methods: We used data on coccidioidomycosis cases reported through the Nationally Notifiable Diseases Surveillance System (NNDSS) in seven states between 1999–2019. We aggregated the data to monthly case counts at the county level and calculated incidence using annual intercensal population estimates from the U.S. Census Bureau. We first used a conservative Box-Pierce white noise test to exclude data from counties that were too sparse or noisy for robust analysis, and we also excluded any counties which had fewer than 200 cases over the whole period. For the remaining counties, we smoothed and de-trended the time series so that relative patterns of incidence could be explored. We used wavelet analyses to extract the significant interannual periods from each of the county time series, analyzed coherence between counties, and examined broader correlations between cycles of incidence across temporal and spatial lags using spline correlograms.

Results: Approximately 40 counties passed the initial filter for further analyses, primarily counties in California and Arizona with high case counts. The time period between consecutive peaks in incidence varied across counties, with a median of 19 months and a range of 12 – 28 months. The specific period lengths identified for each county were sensitive to how the time series were detrended, but consistently ranged from 1 – 2 years. When comparing between counties, relative changes in incidence (i.e., whether incidence was increasing, decreasing, or peaking relative to recent months) were correlated in time (at up to approximately a three-month lag) and space (i.e., for counties up to ~250 km away from each other).

Conclusion: We found that peaks in coccidioidomycosis incidence occurred every one to two years. The time between peaks was not consistent across counties, and in some cases changed across the time series. In the future, we plan to evaluate how covariates such as timing of precipitation and drought, soil humidity, and other spatiotemporal factors can predict the relative patterns in incidence and how they are correlated with each other across time and space.

COCCIDIOIDOMYCOSIS IN THE VETERANS HEALTH ADMINISTRATION (VHA), 2010-2021

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Introduction: The incidence of coccidioidomycosis (CM) has increased in recent years, but there is little data about CM in Veterans. Herein, we describe the epidemiology of CM in VHA during 2010-2021.

Methods: CM-coded hospitalizations (including those with a COVID-19 diagnosis during the same hospitalization) and outpatient visits, as well as Coccidioides culture results were obtained from VHA's Praedico Public Health Surveillance System (1/1/2010-8/31/2021). Data extracted included patient demographics, location, diagnosis codes, encounter details and deaths during CM-coded hospitalizations.

Results: A total of 6,878 unique patients were identified. Of these, 42 were identified by culture result only and had no CM-coded encounters during this time period. Median age at first CM encounter was 64 years (range 18-99), and 93% (6,392) were male. Race was 69.5% White, 15.8% Black/African American, 1.3% American Indian/Alaska Native, 3.5% Asian or Pacific Islander, 1.1% Mixed Race, and 8.7% missing. 9.5% were of Hispanic/Latino ethnicity. Nearly 70% (4,779) resided in HHS Region 9 (AZ, CA, HI, NV and Pacific territories), with the top counties of residence being Maricopa, AZ (1,285), Pima, AZ (989), Los Angeles, CA (315), Pinal, AZ (290), and Kern, CA (201). For 3,034 recorded hospitalizations (1,926 unique individuals), median stay was 5 days, with 513 (17%) admitted to an intensive care and there were 135 deaths during a CM-coded hospitalization (4.4%). Approximately 9% of CM-coded hospitalizations from March 2020-August 2021 were also coded with COVID-19 and 18% of those patients died during their hospitalization. There were 41,840 CM outpatient visits recorded (5,901 unique individuals). Hospitalizations and outpatient visits for 2010-2020 increased during the period evaluated and ranged from 186-323 admissions and 2,751-5,003 outpatient visits annually.

Limitations: Case finding was based on diagnosis codes and/or culture results but did not include other testing modalities. Therefore, cases are not necessarily laboratory-confirmed and we may have missed other cases that were laboratory diagnoses only. Veterans that were evaluated or treated at non-VA locations may not have been captured. Additionally, we did not assess for exposures or environmental/occupational risk factors related to CM.

Conclusions: CM causes substantial morbidity and mortality in Veterans with cases occurring primarily in AZ and CA. The number of VHA encounters and hospitalizations for CM has increased in recent years. More study is needed to determine whether patients co-infected with CM and COVID-19 are at higher risk for severe disease.

INFLUENCE OF METEOROLOGICAL FACTORS AND DROUGHT ON COCCIDIOIDOMYCOSIS INCIDENCE IN CALIFORNIA, 2000–2020

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Background: Coccidioidomycosis is an emerging infection in the southwestern United States. We examined the effects of precipitation and temperature on the incidence of coccidioidomycosis in California during 2000-2020, and estimated incident cases attributable to the California droughts of 2007-09 and 2012-15.

Methods: We analyzed monthly California coccidioidomycosis surveillance data from 2000–2020 at the census tract-level using generalized additive models. Models included distributed lags of precipitation and temperature within each endemic county, pooled using fixed-effects meta-analysis. An ensemble prediction algorithm of incident cases per census tract was developed to estimate the impact of drought on expected cases.

Results: Across 14 counties examined, coccidioidomycosis was strongly suppressed during, and amplified following, the 2007-2009 and 2012-2015 droughts. An estimated excess of 1,358 and 2,461 drought-attributable cases were observed in California in the two years following the 2007-2009 and 2012-2015 droughts, respectively. These post-drought excess cases more than offset the drought-attributable declines of 1,126 and 2,192 cases, respectively, that occurred during the 2007-2009 and 2012-2015 droughts. Across counties, a temperature increase from the 25th to 75th percentile (interquartile range) in the summer was associated with a doubling of incidence in the following fall (incidence rate ratio (IRR): 2.02, 95% CI: 1.84, 2.22), and a one IQR increase in precipitation in the winter was associated with 1.45 (95% CI: 1.36, 1.55) times higher incidence in the fall. The effect of winter precipitation was stronger (interaction coefficient representing ratio of IRRs: 1.36, 95% CI: 1.25, 1.48) when preceded by two dry rather than average winters. Incidence in arid lower San Joaquin Valley counties was most sensitive to winter precipitation fluctuations, while incidence in wetter coastal counties was most sensitive to summer temperature fluctuations.

Conclusions: In California, wet winters along with hot summers, particularly those following previous dry years, increased risk of coccidioidomycosis in California. Drought conditions may suppress incidence, then amplify incidence in subsequent years. With anticipated increasing frequency of drought in California, continued expansion of incidence, particularly in wetter, coastal regions, is expected.

ELUCIDATING THE INTERACTIONS BETWEEN MACROPHAGES AND *COCCIDIOIDES*

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Introduction: Coccidioidomycosis or Valley Fever is a fungal infection caused by *Coccidioides* spp. with a wide range of outcomes of infection, from asymptomatic infection to meningitis, yet the host and fungal factors that underlie these differences remain largely unknown. We are investigating the role of innate immune cells in the early host response to infection, specifically the role of macrophages and G-protein coupled receptor C3aR1 in host response to *Coccidioides* arthroconidia.

Methods: Bone marrow derived macrophages were isolated from wildtype (C57BL/6) or C3aR1-/- mice. *Coccidioides posadasii* Silveira arthroconidia were used in all experiments. Macrophage phagocytosis of arthroconidia was examined by confocal microscopy using a dual staining approach, incubating macrophages with FITC-labelled arthroconidia at a multiplicity of infection (MOI) of 1 (1 arthroconidia for every macrophage) and at each time point labeling extracellular arthroconidia with Calcofluor White. To evaluate the ability of arthroconidia to transition to spherules, the host parasitic form, in the presence of macrophages, we incubated arthroconidia with macrophages at MOI 0.1 (1 arthroconidia for every 10 macrophages) or MOI 1 and examined fungal morphology by light microscopy over 72hrs. RNAseq was performed on RNA isolated from macrophages infected with *Coccidioides* arthroconidia at MOI 1 or MOI 0.1 at multiple timepoints (1hr, 24hrs, and 48hrs). All experiments were conducted at 37C and 5% CO2.

Results: We show that by 1 hr of infection, half of all macrophages had intracellular arthroconidia, with phagocytosis occurring as quickly as 15 min. Interestingly, early phagocytosis is dependent on the host complement 3a receptor (C3aR1), which our laboratory has shown is necessary for efficient phagocytosis of fungi by macrophages (https://www.biorxiv.org/content/10.1101/2021.12.30.474615v1). In macrophages lacking C3aR1, 10% of macrophages had phagocytosed arthroconidia compared to 45% in wildtype cells at 30 min. We next observed that the presence of macrophages strongly promoted the ability of arthroconidia to transition to spherules at temperatures that would not normally promote significant spherulation in vitro. Small spherules were observed within macrophages, in addition to larger extracellular spherules. Preliminary RNAseq data showed minimal changes in transcription at 1hr, with significant upregulation of macrophage genes associated with acute inflammation at 24hrs and 48 hrs, including targets of the well-known regulator NFκB.

Conclusions: This work shows that macrophages phagocytose arthroconidia, yet in the presence of macrophages, some arthroconidia are able to develop into the pathogenic host form of *Coccidioides*. We have created a foundation for better understanding the initial interactions between key host immune cells and the inhaled form of *Coccidioides*.

DETECTION OF *COCCIDIOIDES* SPP. ON THE CHANNEL ISLANDS, CALIFORNIA

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INTRODUCTION: In coastal California, sea lions and other marine mammals, have occasionally been diagnosed with coccidioidomycosis, caused by the soil-borne fungal pathogen *Coccidioides* spp. which is endemic to the San Joaquin Valley of California and other areas in the Southwestern U.S. Though the presence of Valley Fever has been identified in numerous regions throughout the state, there has never been an attempt to detect *Coccidioides* on the Channel Islands which receive a substantial amount of dust deposits yearly from the mainland.

METHODS: A soil sampling plan (5-10 cm depth) was developed for Catalina Island, being the first island to be investigated, using information obtained from the United States Department of Agriculture (USDA) web soil survey database. Soil samples from areas near Avalon (n=36) and Two Harbors (n=42) were collected and analyzed for *Coccidioides* using a nested Polymerase Chain Reaction (PCR) approach. We are planning to expand this research by including soils from San Clemente Island and San Miguel Island in the future.

RESULTS: Out of 78 soil samples analyzed so far, several were positive for *C. immitis* (Avalon, n=1) and *C. posadasii* (Two Harbors, n=11).

CONCLUSION: Our results show that *Coccidioides* can be found in soils on Catalina Island. However further research needs to be completed to investigate if the pathogen is established on the island or if its presence can be explained by dust (or soil) deposits. The presence of *Coccidioides* spp. on the Channel Islands might pose a risk for humans vacationing on the island as well as terrestrial and marine mammals that can be found on or in the waters around the islands.

FINDING LOVE IN DESPERATE CONDITIONS: SEX AS A RESPONSE TO NUTRIENT LIMITATION IN *COCCIDIOIDES POSADASII*

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INTRODUCTION: Despite genetic evidence that sexual recombination likely occurs in *Coccidioides* spp. in the environment (Burt et al., 1996; Mandel et al., 2007) and the presence of unique structures produced in response to compatible mating types (Orr, 1968; Sigler et al., 1998), to date, no one has reliably produced and imaged structures associated with sexual recombination in *Coccidioides* spp. until now (Figure 1).

METHODS: 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 on different concentrations of nutrient availability.

RESULTS: Preliminary data show that in nutrient dense conditions, *Coccidioides posadasii* isolates undergo hyphal fusion (plasmogamy) but do not undergo the process of ascus and ascospore development. However, in nutrient-limited conditions, compatible fungi not only undergo plasmogamy and potential karyogamy, they also undergo the process of ascus and ascospore maturation within a cleistothecium-like structure that hardens and protects ascospores from adverse environmental conditions.

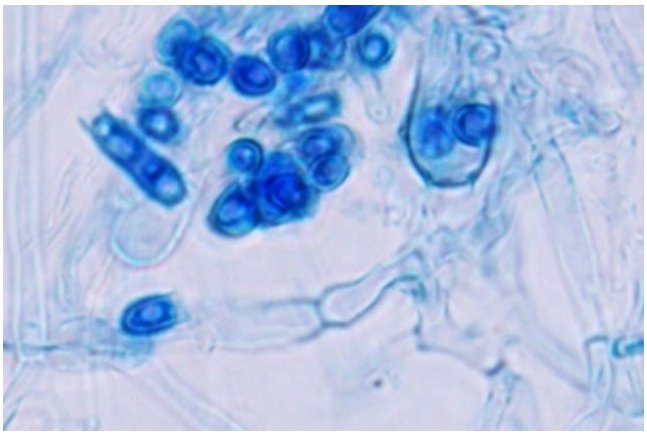

Figure 1 Mating structures of *Coccidioides posadasii* in the absence of Nitrogen or Carbon sources. Within the image include the vase-like vessels (asci) which contain meiospores (ascospores) and hyphal fusion (plasmogamy).

CONCLUSION: Viable ascospore production in response to nutrient-limited environments speaks to the possibility of climate change leading to novel variants of *Coccidioides* and may explain, in part, the recent rise in Valley Fever infections seen in Arizona and California (Centers for Disease Control and Prevention, 2021). Additionally, the hardiness of the cleistothecia produced during the process of sexual recombination may explain infections from areas not actively associated with animal burrows thus lending support for the endozoan, small-mammal reservoir hypothesis proposed by Taylor and Barker (2019).

ESTABLISHMENT OF A *GALLERIA MELLONELLA* MODEL FOR THE STUDY OF VIRULENCE AND ANTIFUNGAL DRUG SUSCEPTIBILITY OF *COCCIDIOIDES*

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Introduction: *Galleria mellonella* has been employed for studying fungal pathogens such as *Candida*, *Aspergillus*, and *Cryptococcus*, but it has yet to be established for dimorphic fungal pathogens (i.e. *Coccidioides spp*). *G. mellonella* larvae possess a robust innate immunity comparable to vertebrates against fungal infections. In this study we evaluate the larva model for characterization of virulence factors and assessment of drug susceptibility against *Coccidioides* infection. Our objective is to establish the infection criteria of *G. mellonella* larvae with *Coccidioides* spores for high throughput screening of virulence factors and potential drug candidates against *Coccidioides*.

Materials and Methods: A clinical isolate of *C. posadasii* (C735) was used in this study in a BSL-3 laboratory. The larvae were purchased from a commercial source and stored at 20°C before use. Larvae (0.15-0.2g) were injected with 5-10x10^5 viable spores in 10 µl PBS by the haemocoel route and incubated at 37°C with 10% CO_2 for the period of the experiment. The larvae were measured for melanization production rates, histopathology, fungal burden and survival. The model then is applied for screening of *Coccidioides* mutants that were created by *Agrobacterium* (Ti plasmid) facilitated random gene disruption. C57BL/6 and BALB/c mice were challenged by the oropharyngeal aspiration with a suspension of potentially lethal dose of spores (450-470) prepared from the parental and the mutant strains. The larva model can also be used for validating newly discovered antifungal compounds *in vivo*. Drugs were administered via the haemocoel injections at a selected dose (1-, 2- and 5-fold) of minimal inhibition concentration (MIC) obtained using *in vitro* spherule cultures at 2, 48 and 96 hr postchallenge. Amphotericin B and PBS served as positive and negative controls, respectively.

Results: In the larva model, *Coccidioides spp.* can covert to parasitic growth and form spherules, the same morphotype in mammalian hosts. Surprisingly, the larvae were relatively resistant to *Coccidioides* infection compared to mice that have a LD_{100} of ~100 spores administrated by the oropharyngeal and intranasal routes. The infected larva survived for 2 and 4 days postchallenge with 1.0 and 0.5 million spores, respectively. Melanization score was peaked at 3 days postchallenge when they were challenged with a dose of 5x10^5 spores and allowed a longer period for evaluation of virulence and drug efficacy. Thus, it was the challenge dose used for subsequent experiments. We have identified 4 *Coccidioides* mutants that lost virulence in the larva model. Further evaluation of the mutants using a murine model of pulmonary coccidioidomycosis confirmed that the mutants were attenuated. Genomic sequence analysis of the mutant strain (*Cp*-30) revealed that a gene encoding for a 244-amino acid protein was disrupted. The *Cp*-30 mutant reduces conversion to parasitic spherules, while it appears to have normal saprobic growth. Furthermore, *in vivo* the *Cp*-30 mutant is highly attenuated in both the larva and murine models of coccidioidomycosis. Experiments are underway to characterize this virulence gene. Furthermore, we employed this larva model for assessing treatment efficacies of newly identified antifungal drugs from Broad Institute Repurposing drug library. Results demonstrated that 2 novel antifungal agents could prolong larva survival in a similar efficacy as Amphotericin B.

Conclusion: We have established a mellonella larva model of *Coccidioides* infection. Our findings suggest that *G. mellonella* is a useful model of coccidioidomycosis and convenient for pre-screening assays for the identification of fungal virulence factors and novel antifungal drugs.

PREDICTION AND DISCOVERY OF *COCCIDIOIDES*-SPECIFIC T CELL EPITOPES

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Introduction: Vaccination against coccidioidomycosis is feasible as patients can develop life-long immunity to VF. Long-term memory to VF requires T helper cell (Th) activation by the major histocompatibility complex (MHCII) molecules expressed in antigen-presenting cells (APCs). Antifungal immunity is associated with mixed Th1- and Th17-type responses. We have used an immuno-bioinformatics platform to screen *Coccidioides* genomes and to predict potential T cells epitopes that are further validated using a human HLA-DR4 (DRB1*0401) transgenic mice and *ex vivo* recall assays using human PBMCs derived from healthy donors and VF patients. Our overall goal is to identify *Coccidioides*-specific epitope that can bind to human MHCII molecules and elicit T-cell mediated immunity.

Methods: We have applied an *in silico* approach using EigenBio software (IoGenetic LLC.) to predict potential Th epitopes from well-characterized *Coccidioides* antigens (Fast track) and from proteins that are highly expressed during the parasitic phase (Parasitic phase track). We first cloned, expressed, and purified recombinant proteins derived from *Coccididoides* and encapsulated them into glucan-chitin-particle adjuvant as a vaccine model. We evaluated T cell reactivity to the predicted synthetic peptide and peptide libraries using IFN-γ ELISA assays. Mice were vaccinated three times with either the full-length protein or adjuvant control then tested for in vitro recall response to synthetic peptides representing the predicted epitopes (15-mer to 25-mers, GenScript). Lymphocytes were isolated from the spleens of immunized HLA-DR4 mice (n= 4-5 mice per group). Furthermore, we utilized an *ex vivo* approach to validate human cell immunity by co-culturing autologous $CD4^+$ T cell and APCs of healthy donors in the MiMIC system (Sanofi) as well as cytokine recall assays in patient samples.

Results: We completed bioinformatics analysis for 10 well-characterized fast-track antigens and ~200 coccidioidal proteins that are shown to be highly expressed in the parasitic phase. We identified 17 IFN-γ-stimulating epitopes from 6 fast-track coccidioidal antigens for the HLA-DR4 allele using the transgenic mice. Currently, 18 of the 20 VF recovering patients have a Stimulation Index greater than 1 and recognize the multivalent antigen (rCpa1), and they are reactive with one or more of the identified epitopes. Parallelly, these antigens and peptides are under evaluation using an *ex vivo* T cell assay platform called MiMIc provided by Sanofi using primed DCs and Th cells isolated from healthy donors. Additionally, we obtained control blood samples from healthy donors outside the historical endemic area. Comparison of IFN-γ expression amounts from the VF patients (n=19) versus the healthy subjects (n=16) is significant ($P < 0.05$).

Conclusions: We have successfully established a bioinformatics prediction in conjunction with *ex vivo* T cell-recall assays to identify short peptides (17-28 aa) of Coccidioides antigens that can stimulate IFN-γ production. These short peptides are deposited in the public immune epitope database (IEDB) that can be used to facilitate the development of diagnostic kits and vaccine antigens.

ASSESSMENT OF SPATIOTEMPORAL DISTRIBUTION OF *COCCIDIOIDES* WITHIN AMBIENT AIR IN PHOENIX, AZ

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Introduction: While aerosolization of *Coccidioides* arthroconidia is a driver of Valley Fever infections, a limited amount of research has focused on characterizing the spatial and temporal distribution of arthroconidia within the ambient air. Where and when *Coccidioides* is airborne is crucial to accurately understand and model the complex dynamics of this endemic pathogen at both a local and regional level.

Methods: Portable air sampling units were used for air sampling across a 24-hour period across 23 sites in the Phoenix metropolitan area. Daily collections over a 30-month period were conducted as part of an ongoing surveillance in collaboration with the U.S. Department of Homeland Security, AZ Department of Health Services and the Centers for Disease Control and Prevention. DNA was extracted from air filters using Qiagen DNeasy PowerLyzer PowerSoil kits and DNAs were screened for *Coccidioides* DNA using previously published molecular techniques.

Results: In total, between TGen and the CDC, 9361 filters were collected from July 2017 through December 2019 and 5480 have been screened for the presence of *Coccidioides*. Across 5465 tested samples, a total of 394 were positive for *Coccidioides* DNA. Of the 23 sites, 5 had no detected positives, and the remaining 18 sites had an average prevalence of 3.7%. One site in particular stood out as a hot spot, with a 21.8% prevalence rate, well above the range of 0.6-6.4% observed in the other positive sites.

Conclusion: Our data suggest an uneven distribution of *Coccidioides* bioaerosols in the Phoenix metropolitan area, ranging from areas with no detected fungus to distinct “hot-spots.” Such findings support our hypothesis that local factors are involved in the aerosolization of arthroconidia and may help drive potentially different disease risk levels within the city. Our next steps aim at examining the association of the supposed drivers and detectable *Coccidioides* within ambient air.

URGENT CARE PRACTICE PATTERNS FOR DIAGNOSING COCCIDIOIDOMYCOSIS IN A HIGHLY ENDEMIC URBAN POPULATION.

Jie Pu, Valerie Miranda, Devin Minior, Shane Reynolds, Benjamin Rayhorn, and John N Galgiani.

INTRODUCTION: We found previously that very few urgent care (**UC**) patients (**pts**) were diagnosed with coccidioidomycosis (**CM**). Since 2020, during onboarding, at quarterly meetings, and in periodic emails, we have encouraged UC clinicians to more frequently test for CM for pts with pneumonia (**PNA**)when appropriate.

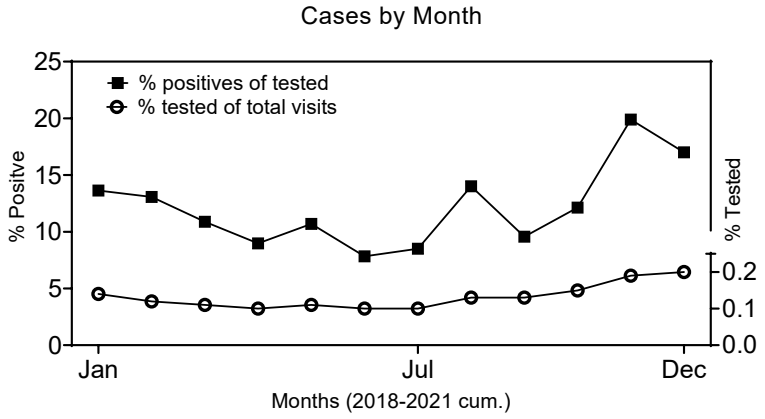
METHODS: Banner UC clinics have increased to n=48 by 2021 with now a staff of over 250 clinicians. Installation and training of a common electronic medical record was completed in 2017. In March 2022, a data download was created for this analysis of UC clinician patterns of coccidioidal serologic testing (**CST**, mostly EIAs), CST results, and their relation to patient ICD10 profiles.

RESULTS: For the years 2018-19 (787K UC visits) and 2020-21 (1,290K UC visits), CSTs were ordered per 10^4 visits 6.1 and 17.8 times, respectively (chi-squared $p<0.0001$). Positive CST were highest for August, November and January (17.0%) and lowest for other months (10.6%). Among ICD10 codes most frequently associated with positive CST visits were PNA (n=187), cough (n=174), fever (n=63), bronchitis/URI (n=43), and *Erythema nodosum* (**EN**, n=27). Of 176 EN pts, only 6 also had PNA. During the study period, pts with PNA had increased CSTs overall, CSTs on the first visit, and CSTs on the second visit when the first visit was negative. For EN pts, 61% had positive CST. With increased clinician reminders, the frequency of CTS of pts with PNA has increased three-fold, but still CST is done in less than three-quarters of pts where recommended.

CONCLUSION: Routine quality improvement activities have significantly but only partially improved rates of testing pts with PNA for CM in UC clinics located in a highly endemic area. Innovative strategies may be needed to improve current practice. Also in our region, EN, independent of PNA, is a strong predictor of CM.

Results	2018	2019	2020	2021
Total UC patients (thousands)	373	414	593	698
Total UC pts with PNA	2,094	2,565	3,473	3,558
PNA pts with CST	7.2%	7.9%	21.1%	22.0%
1st visit # tested (% pos.)	21 (29%)	45 (29%)	478 (20%)	543 (13%)
2nd visit # tested (% pos.)	129 (14%)	157 (18%)	254 (31%)	238 (16%)
Both visits # tested (% pos.)	2 (100%)	3 (0%)	39 (41%)	45 (62%)
PNA pts with positive CST	17.3%	20.8%	26.0%	17.5%
Total UC pts with EN	21	44	58	53
EN pts with CST	9.5%	25%	24%	32%
EN pts with positive CST	50%	55%	71%	59%

Figure. Frequency of testing for CM in all visits and percentage of tests that were positive in BUCS by month for the years 2018-2021.



COCCIDIOIDOMYCOSIS OF THE SPINAL CORD - ANALYSIS OF 19 CASES AND REVIEW OF THE LITERATURE

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Introduction: Central nervous system involvement with coccidioidomycosis is a serious infection that is universally fatal if not treated. Prior to the advent of MRI, very few reports described spinal cord involvement and autopsies often omitted spinal cord examination. Accurate localization of the anatomic location affected by CNS cocci at initial diagnosis can be challenging due to mental status changes and the presence of brain abnormalities. At the Valley Fever Institute we adopted the practice of performing MRI imaging on the entire neuro-axis at the time of diagnosis. Here we describe the radiologic and clinical characteristics of 19 cases of CNS cocci who had spinal MRI imaging performed

Methods: This study was approved by Kern Medical 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. Patients were included if they qualified for the diagnosis of probable CNS coccidioidomycosis and had Magnetic Resonance Imaging performed on their spinal cord

Results: The majority of patients studied had abnormal spinal imaging. Arachnoiditis being the most common, followed by myelitis, spinal abscess and syringomyelia. Interestingly, spinal cord abnormalities can be asymptomatic. Most common symptom was back pain followed by radiculopathy

Conclusion: Coccidioidal meningitis frequently involves the spinal cord. Radiologic findings include leptomeningeal enhancement, adhesive arachnoiditis with nerve root clumping, myelitis and syringomyelia. Heightened awareness is required due to unpredictable symptomatology

STRUCTURAL AND FUNCTIONAL STUDIES OF *COCCIDIOIDES* CPS1

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INTRODUCTION: A live-attenuated coccidioidomycosis canine vaccine has been developed based on a *Coccidioides posadasii* Silveira mutant with a *CPS1* gene deletion. The mutant strain fails to produce mature spherules and is avirulent even in immunodeficient mice indicating it's safety. To understand why the *CPS1* deletion is so debilitating, we are defining the structural and functional domains of the protein the role of *CPS1* in normal growth and spherulation of *Coccidioides*.

METHODS: To define the role the domains Cps1 have in spherulation, derivatives of *C. posadasii* strain Silveira expressing domain deletion derivatives of *CPS1* were created and screened for virulence in a mouse infection model and for in vitro spherulation. An avirulent Δ*cps1*::Bleo strain was created to be used as a recipient for mutated *CPS1* genes. Three domain deletion constructs of *CPS1* were created, each with a deletion of a single conserved domain, the 67 amino acid DMAP1b domain, or one of the two adenylate-forming domains, AMP1 (302 amino acids) or AMP2 (452 amino acids). Gene constructs were tagged at the C-terminus with in-frame c-myc and His tags for validation of protein expression. The domain deletion constructs, containing the *hphR* gene were transformed into the Δ*cps1::Bleo* strain selecting for hygromycin resistance. Transformed strains were purified and screened by antibiotic resistance, PCR and DNA hybridization to confirm insertion of the gene deletion construct at the *CPS1* locus by replacement of the *BleoR* marker. Virulence studies were performed using C57BL/6 mice (8 mice per strain) with lung and spleen fungal burdens compared by Kruskal-Wallis. To demonstrate expression of the *CPS1* domain deletion proteins, Westerns were performed on protein extracts and analyzed by c-myc antibody screening.

Protein prediction programs have suggested that Cps1 is a transmembrane protein. For structural characterization of Cps1, *CPS1* cDNA copies containing an N-terminal FLAG tag and a C-terminal His tag, were expressed in *Saccharomyces cerevisiae* plasmid 83nu under the control of the inducible *GAL1* promoter. Protein purification was performed by lysis of induced cells followed by membrane purification and extraction with different detergents. Efficiency of protein solubilization following detergent extraction was determined by Western analysis of membrane supernatant and pellet fractions using anti-FLAG or anti-His antibodies. The structure of Cps1 was analyzed recently using AlphaFold2.1, the AI-based protein structure prediction program developed by Google's Deep Mind.

RESULTS: Deletion of either of the two Cps1 adenylate-forming domains, AMP1 or AMP2, resulted in strains that reiterated the Dcps1 phenotype; they were avirulent and failed to persist in mice. Deletion of the Cps1 DMAP1b domain produced strains that were fully virulent in mice. Western analysis demonstrated that the proteins were expressed in the transformed strains. Although previous protein structure programs predicted Cps1 contains between four and seven transmembrane domains, AlphaFold2.1 predicts that Cps1 lacks transmembrane domains and is a globular peripheral membrane protein. The Cps1 globular prediction indicates positively charged patches of amino acids complimentary to the negative head groups of membrane lipids. Recombinant Cps1 shows limited solubilization from the membrane fraction using a variety of membrane solubilization detergents. In contrast, a high salt (1 M NaCl) extraction without detergents resulted in recovery of the highest levels of Cps1. AlphaFold2.1 also predicts a conserved ATP binding domain that corresponds to part of the AMP2 domain in Cps1.

CONCLUSION: Mouse studies and in vitro spherulation demonstrate that the two catalytic adenylation domains of Cps1 are critical for spherulation and virulence, while the DMAP1 binding domain is dispensable. This indicates that the catalytic activity of Cps1 is important for Cps1 function. Predictions and preliminary binding studies connect the catalytic activity of AMP2 with ATP binding. Understanding the targets of Cps1 that result in the profound spherule defect of *CPS1* mutants will aid our understanding of parasitic phase development.

PROTECTIVE HOST RESPONSES AGAINST *COCCIDIOIDES**

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*Did not wish to publicly release abstract

INTERACTIONS BETWEEN VALLEY FEVER AND GENETIC ANCESTRY: DOES GENETIC ANCESTRY AFFECT RISK OF DEVELOPING DISSEMINATED COCCIDIOIDOMYCOSIS?

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INTRODUCTION: Coccidioidomycosis, commonly known as "Valley Fever," is an endemic fungal infection in the Southwest part of America. The majority of patients are asymptomatic after infection or develop only a respiratory infection. However, a rare fraction of individuals go on to develop severe disseminated coccidioidomycosis (DCM), which is associated with significant morbidity and mortality and requires prolonged and sometimes lifelong antifungal treatment. The factors that contribute to an individual's risk for developing DCM are multifactorial, however, the genetic basis of this risk remains unclear.

METHODS: Both rare and common genetic variants have been shown to contribute to infection severity in a variety of infectious diseases, including herpes encephalitis, COVID-19 and tuberculosis. Early epidemiological studies for coccidioidomycosis have also identified an association of self-identified race and ethnicity with the risk of DCM. We obtained whole exome sequencing data from 479 individuals with coccidioidomycosis from the UC Davis Center for Valley Fever.(468 individuals after quality control). This cohort includes a mixture of both severe and mild coccidioidomycosis, including 109 individuals who developed DCM. We also obtained whole genome sequencing from 87 individuals with coccidioidomycosis and blood RNA-seq data for a subset of this group (n= 42) from the Valley Fever Institute at Kern Medical. We plan to use these data to validate our preliminary results from the exome data and investigate expression and splicing differences associated with genetic ancestry. We determined genetic ancestry through PCA analyses then ran ADMIXTURE mapping with k=4 to determine global ancestry proportions. We then calculated odds ratios of DCM compared to what we classify as Uncomplicated Valley Fever (UVF) dependent on global ancestry proportions.

RESULTS: We find through PCA analyses that patients with African genetic ancestry have an increased chance of having DCM in our cohort. To further investigate this signal, we looked at global ancestry proportions. Using unsupervised ADMIXTURE mapping we found that the global ancestry of our exome patients can be divided into 4 subgroups.

The first subgroup, k1, is closely associated with African genetic ancestry. 95% of the individuals who were identified as being of African genetic ancestry through PCA analyses (63, or 13.5% of our cohort) had over 50% of their global ancestry represented by k1, and no patients with over 50% of their global ancestry represented by k1 were identified as not being of African genetic ancestry. Odds ratio analyses show that individuals with 100% k1 global ancestry had 17 odds of DCM vs UVF (p=1.7e-12).

The fourth subgroup, k4, is closely associated with European genetic ancestry. 100% of the 162 individuals who were identified as being of European genetic ancestry through PCA analyses had over 63% of their global ancestry represented by k4. An additional 23 patients had over 50% of their global ancestry represented by k4, bringing the total number of our cohort with over half k4 global ancestry to 185, or 40% of our cohort. Odds ratio analyses show that individuals with 100% k4 global ancestry had 0.25 odds of DCM vs UVF (p=9.7e-7).

Thus, we show through ADMIXTURE mapping and an odds ratio analysis that the k1 portion of the genome, which is associated with patients of African genetic ancestry, carries an increased risk of DCM, while the k4 portion of the genome, which is associated with patients of European genetic ancestry, carries a decreased risk of DCM. In the future, we plan to do supervised ADMIXTURE analyses as well as local ancestry analyses to further investigate these results. We also plan to validate our results with the 87 genomes from the Valley Fever Institute. RNAseq from some of the genome-sequenced individuals (n=42) will be used to interrogate the pathways that explain this association.

CONCLUSION: Our work described here confirms that African genetic ancestry is an independent risk factor for DCM status in this specific cohort. Future work will investigate whether this is replicated in an independent cohort from the Valley Fever Institute. This project has the potential to help us develop genetic biomarkers to identify individuals at the highest risk for DCM and those who would benefit from early treatment with immunomodulatory therapies.

SERUM PROCALCITONIN LEVEL IN PULMONARY COCCIDIOIDOMYCOSIS INFECTION

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Introduction: Procalcitonin, the peptide precursor of calcitonin, was first studied as a biomarker in acute severe bacterial infections in 1993 and has been deemed helpful in differentiating between bacterial and viral infections. Procalcitonin has been useful as an adjunct to clinical judgment for guiding antibiotic therapy and its discontinuation. It has been best studied in distinguishing between viral and bacterial lower respiratory infections. The relationship between serum procalcitonin levels and primary coccidioidomycosis was initially studied by Sakata et al. in 2014 and did not reveal a relationship between elevated procalcitonin and coccidioidal infection. The purpose of this study was to determine any association between serum procalcitonin levels and primary pulmonary coccidioidomycosis.

Methods: We conducted a retrospective chart review study using the Valley Fever Institute database between 2017 and 2021. This study was approved by the Kern Medical Institutional Review Board. The literature search was conducted on PubMed and Google scholar using coccidioidomycosis; community-acquired pneumonia; procalcitonin levels as keywords. Coccidioidomycosis infection was confirmed by serology, or microbiology of sputum or broncho-alveolar lavage, and radiological evidence of pneumonia. Bacterial infections were excluded by reviewing the results of sputum and blood cultures.

74 patients were enrolled during in-patient care. We identified 52 patients with acute infection and 22 patients with chronic infection. Acute infection was defined as new symptomatic primary pulmonary coccidioidomycosis of < 6 weeks' duration. Chronic infection was defined as either proved previous coccidioidomycosis infection or pneumonic symptoms of ≥ 6 weeks' duration. The first value of the procalcitonin assay, with a cutoff of > 0.10 μg/L being positive.

Results: Of all patients with acute infection 34 (65.38%) had a positive test for Procalcitonin as compared to 12 (54.54%) for the Chronic patients. The odds ratio is 1.57 suggesting a greater incidence of positive procalcitonin among acute patients; however, the finding is not statistically significant ($p = 0.3811$).

Conclusion: This study did not find the clinical value of procalcitonin in the diagnosis of pulmonary coccidioidomycosis or differentiating acute from chronic infection. Procalcitonin does not distinguish bacterial pneumonia from coccidioidal pneumonia. Further larger randomized controlled studies are needed to investigate this relationship. Clinicians should continue to use clinical judgment and laboratory as well as imaging to distinguish pulmonary coccidioidomycosis vs. bacterial pneumonia.

IMPACT OF VALLEY FEVER ON QUALITY OF LIFE

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INTRODUCTION: Valley Fever (VF) infection can cause significant chronic disease. Impact on quality of life is poorly understood across disease severity.

METHODS: An IRB approved survey was designed by VF care providers at the Valley Fever Institute (VFI), UCLA, and UCSF. After obtaining informed consent, the survey was performed during routine in-person visits at the VFI from March 2020 to March 2021. Major topics in the questionnaire included quality of life, social determinants of health, and barriers to receiving treatment. VF infection category, and VF infection type were compared across survey results and demographics using Fisher's exact test and Chi-squared tests.

RESULTS: The survey was administered at the VFI to 83 adult participants with a mean age of 45.9 years. 53.2% were male, 69.6% Hispanic/Latino, 20.3% white, 3.8% African American/black, 2.5% Asian, and 2.5% Pacific Islander. Cohort VF category and infection type are summarized in Table 1.

Table 1. Participant Valley Fever Classifications

Classification	(%)
VF Category (n=76)	
Complicated VF	47.4%
Uncomplicated VF	52.6%
VF Infection Type (n=79)	
Mild pulmonary	55.7%
Severe pulmonary	5.1%
Meningitis	32.9%
Single skeletal	7.6%
Multi-bone disease	5.1%
Other	7.6%

41.7% (n=35) of participants reported hospitalizations within the past 12 months, with 57.1% (n=20) of these hospitalizations related to their valley fever. 51.2% (n=42) report experiencing at least moderate fatigue over the past seven days. 41.3% (n=33) rate their physical health as fair or poor. 48.2% (n=39) report at least moderate limitation to their normal physical activity due to VF. Similarly, 35.1% (n=28) experience at least frequent work difficulties. 53.8% (n=43) agree VF has prevented them from maintaining employment. Of those currently not working 63% (n=51), 62% (n=31) report cause as due to illness/ disability. Four (4.9%) participants revealed there are barriers to receiving treatment for VF and cited financial, insurance issues and provider lack of knowledge as factors. Males had significantly higher job losses from VF ($p=0.011$). Females reported significantly worse mental health ($p=0.016$). Participants with complicated VF infection report worse quality of life ($p=0.020$), lower satisfaction with their social activity ($p=0.063$), decreased ability to carry out their day-to-day activities ($p=0.065$). Participants with mild pulmonary type VF report significantly better general health ($p=0.013$), mental health ($p=0.032$), overall quality of life ($p=0.0026$), improved ability to carry out their day-to-day activities ($p=0.024$) and higher satisfaction with their social activities ($p=0.034$).

CONCLUSION: This survey characterizes the impact of VF on quality of life across highlights the importance of psychosocial support to ensure patient health and quality of life can be optimized.