PROCEEDINGS OF THE 64TH COCCIDIOIDOMYCOSIS STUDY GROUP

The 64th annual meeting was scheduled for April of 2020.

The in-person meeting was cancelled due to the pandemic,
but the program had already been reviewed and established.
All abstracts selected for presentation are contained herein.

BICAVITARY EOSINOPHILIC EFFUSION IN A DOG WITH COCCIDIOIDOMYCOSIS

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Abstract

INTRODUCTION: Eosinophilic effusions due to coccidioidomycosis have been reported in the human literature, are most commonly found in the pleural cavity, and are thought to result from direct invasion from a lung parenchymal lesion. Eosinophilic effusions are uncommon in veterinary medicine, with the majority of reported cases being secondary to underlying neoplasia.

MATERIALS, METHODS, AND RESULTS: This is a case of coccidioidomycosis in a dog, who was presented for evaluation of vomiting and labored breathing. Physical examination and thoracic and abdominal imaging revealed pleural and peritoneal effusions, which exhibited neutrophilic inflammation with a substantial eosinophilic component. Because neoplasia was considered unlikely and the dog had positive IgM and IgG coccidioidomycosis titers, the eosinophilic component of the inflammation was attributed to coccidioidomycosis infection. The dog was treated with fluconazole and a tapering course of prednisone. Effusions from both body cavities resolved after 6 weeks of anti-fungal therapy. Therapy with fluconazole was discontinued after 12 months of treatment because of resolution of clinical signs and negative IgM and IgG coccidioidomycosis titers. The dog developed a mid-diaphyseal lytic and proliferative lesion in the left radius 3 months after discontinuation of fluconazole. Fine-needle aspiration of this lesion yielded fungal spherules consistent with Coccidioides spp., confirming the presumptive diagnosis.

CONCLUSION: This case illustrates the importance of consideration of coccidioidomycosis as a differential diagnosis when an eosinophilic cavitary effusion is present in dogs that live in or have traveled to endemic regions.

COCCIDIOIDOMYCOSIS AND PULMONARY EMBOLI

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Abstract

INTRODUCTION
Coccidioidomycosis is endemic in California. The organism, *Coccidioides immitus,* usually produces pneumonia but may disseminate resulting in a variety of complications. In 2019, and early 2020, we encountered five patients with coccidioidomycosis who were documented to have pulmonary emboli. We had not encountered such an association over decades of practice within our community, which has had a fairly high yearly incidence of coccidioidomycosis cases.

MATERIALS AND METHODS
Case Series.

RESULTS
Three of the five cases were recognized at the time of referral to our Hematology Clinic for consultation regarding duration of anticoagulation. One case was diagnosed following referral for Infectious Disease consultation and another during hospitalization for pneumonia. Three cases were diagnosed with pulmonary emboli at the time of presentation to an emergency room with complaints of fever, cough and dyspnea, but all had been ambulatory. The remaining two cases had been hospitalized for several days, one with coccidioidomycosis meningitis and the other with persistent fever and an increasing oxygen requirement. All images, four chest CT angiograms and one chest CT with contrast, were reviewed and the presence of pulmonary emboli confirmed.
A detailed literature search failed to locate similar cases. A search of the electronic medical records of a nearby hospital found no cases having both coccidioidomycosis and pulmonary emboli. Additionally, in our discussions with other clinicians, many with long experience in managing coccidioidomycosis, none could recall ever seeing venous thromboembolic disease complicate this infection.

CONCLUSION
A venous thromboembolic complication of coccidioidomycosis is very rare. We encountered five cases within a relatively short period of time. We have no evidence that would illuminate the cause of venous thromboembolism in these patients. We report these cases with the intent of making those caring for patients with coccidioidomycosis, or those interested in the organism and host response to it, aware of these events. By highlighting this relationship, we hope others will look closely at similar cases and help to elucidate the explanation behind this association.

3

SYMPTOMS IN COCCIDIOIDOMYCOSIS VERSUS OTHER RESPIRATORY ILLNESSES IN COMMERCIALLY INSURED ADULT OUTPATIENTS, UNITED STATES, 2016–2017

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Abstract

INTRODUCTION

Coccidioidomycosis causes various symptoms and syndromes, often resulting in confusion with other fungal diseases and illnesses such as bacterial or viral community-acquired pneumonia (CAP), influenza, and tuberculosis. Previously, rash, myalgia, and higher fatigue scores have been the only clinical features that differentiated coccioidal CAP from CAP caused by other pathogens. Few other data directly compare symptoms in coccidioidomycosis with other respiratory infections but could help inform whether certain features could help increase clinicians' suspicion for coccidioidomycosis.

MATERIALS AND METHODS

We used the IBM® MarketScan® Research Databases to identify adult outpatients with International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM) diagnosis codes during 2016–2017 for coccidioidomycosis, blastomycosis; histoplasmosis; viral, bacterial, *Streptococcus pneumoniae*, and unspecified pneumonia; influenza; tuberculosis; and other lower and upper respiratory infections. We compared symptoms on and in the 90 days before diagnosis between these cohorts and examined pre-index date diagnoses among fungal disease patients.

RESULTS

Coccidioidomycosis resembled other pneumonias for most pulmonary symptoms, but cough was more common in coccidioidomycosis (31.4%) vs. influenza (20.2%), blastomycosis (13.1%), and histoplasmosis (14.0%). Fever was more common in coccidioidomycosis (9.4%) than in blastomycosis (2.6%) or histoplasmosis (5.3%), but less common than in influenza (18.5%) and pneumonia (>12.6%) patients. Malaise/fatigue (15.3%) and joint pain (12.9%) were more common with coccidioidomycosis than with any other disease. Solitary pulmonary nodule (13.7%), enlarged lymph nodes (4.4%), hyperhidrosis (1.8%), and erythema nodosum (1.5%) were particularly suggestive of coccidioidomycosis, and unspecified pneumonia was a common pre-index date diagnosis in 16.7% of patients.

CONCLUSION

Coccidioidomycosis more closely resembled other pneumonia etiologies than influenza, so the common descriptor "influenza-like illness" may not be accurate. Coccidioidomycosis appeared to be under-detected and may be difficult to clinically distinguish from other causes of pneumonia except when certain uncommon symptoms are present. This indicates a need for improved detection methods and greater healthcare provider awareness supported by more accurate and focused messaging about its symptoms and when to consider testing patients for coccidioidomycosis.

4

A CASE OF DUAL FUNGAL MENINGITIS

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Abstract

Fungal meningitis are common opportunistic infections among uncontrolled HIV infected population. However, meningitis with two different fungi simultaneously is a rare phenomenon. We report a case of meningitis with concomitant coccidioidomycosis and Cryptococcus neoformans in an HIV positive individual.

A 63 year old male with a remote history of pulmonary coccidioidomycosis and uncontrolled HIV presented to our facility with three weeks of worsening headaches and neck stiffness. Three months prior to admission, his CD_4 was 46 cells/uL and viral load was 220,000 copies/mL; at which time his antiretroviral medications were restarted. Upon admission, lumbar puncture revealed hazy cerebrospinal fluid (CSF) with an opening pressure of 22 cm H_2O. Fluid analysis showed white blood cells of 181 (neutrophilic 58% and lymphocytes 35%), glucose of 19 mg/dL and protein of 471 mg/dL. CSF Cryptococcus PCR was positive and cultures recovered Cryptococcus neoformans. CSF Cryptococcus antigen was 1:512. Brain MRI also showed abnormal leptomeningeal enhancement. Given his prior history of pulmonary coccidioidomycosis, a CT chest was obtained which revealed a right lower lobe cavitary infiltrate with scattered small nodules. Serologic tests for coccidioidomycosis showed immunodiffusion (ImmDiff) positive for IgG with complement fixation (CF) titer of 1:64. CSF coccidioidal ImmDiff was also positive for IgG with CF titer of 1:4. The patient received two weeks of induction therapy with amphotericin and flucytosine followed by fluconazole consolidation therapy. CSF cultures remained negative. No clinical evidence of immune reconstitution inflammatory syndrome was identified. At three month follow up visit, the patient had improving fungal serologic titers and resolving neurologic deficits.

To the best of our knowledge, concomitant fungal meningitis has never been reported in the literature. This case illustrates the successful treatment of dual fungal meningitis with Cryptococcus and coccidioidomycosis in an uncontrolled HIV infected individual.

Immunocompromised individuals are at increased risk of opportunistic infections. Co-infections at the same site are possible; therefore, we remind clinicians to be vigilant in their investigation among at risk populations.

5

NIKKOMYCIN Z (NIKZ) AGAINST DISSEMINATED COCCIDIOIDOMYCOSIS IN A MURINE MODEL OF SUSTAINED RELEASE DOSING

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Abstract

Introduction. It has been shown that *Coccidioides* is highly susceptible to inhibition and killing by nikZ (a chitin synthase inhibitor) *in vitro,* that nikZ is not toxic at high doses in mice, and that nikZ is efficacious in models of coccidioidal meningitis. We assayed nikZ efficacy in disseminated coccidioidomycosis (in a reduction of CFU design), and whether sustained release, in lieu of repeated dosing, could be useful.

Method. CD-1 mice in groups of 10 were challenged intravenously with 193 CFU. One was treated daily with 100 mg/kg fluconazole (FCZ) (gavage), 3 given nikZ IP BID with 100, 300 or 1000 mg/kg; 3 given nikZ PO 200, 600 or 2000 mg/kg daily in their drinking water; 1 untreated (PO water control), and one given mock doses with water IP. Treatments were started day 4 after infection, continued for 5 days; 3 days later mice were euthanized and organ burdens quantitated. Sustained release was mimicked by matching drinking throughout the day to assure each day's dose was taken, and that mice always had water.

Results. All mice survived, showed no aversion to medicated water, maintained normal behavior; IP nikZ treated mice were apathetic and had ruffled fur for 1 hour after doses.
Resulting infection (mean CFU burden/organ) was as follows:

Organ	FCZ	Water	nikZ 200 IP	nikZ 600 IP	nikZ 2000 IP	Water	nikZ 200 PO	nikZ 600 PO	nikZ 2000 PO
Lung	948.6	803,047	0.5	0	0	980,745	4.2	0	0.5
Liver	311.4	593,288	0	0.7	1.4	84,913	0	0	0
Spleen	9.7	146,390	0	0	0	249,047	0.5	0	0.5

In all 3 organs, all nikZ-treated (at all doses) burdens were less ($p<0.001$) than their respective controls, and less than the FCZ-treated. In addition, 6, 2 or 9 FCZ-treated were sterilized in lung, liver and spleen, respectively, whereas 9 or 10 nikZ-treated in every treatment group in every organ were sterilized, with the exception of liver, 2000 IP dose, and lung, 200 PO dose, where 8 and 7, repectively were sterilized.

Conclusion. nikZ is highly efficacious against disseminated coccidioidomycosis, in all organ systems. The mimic of sustained release PO, which would give a steady AUC, suggests efficacy equal to assured total dosing parenterally twice daily.

COMPARATIVE STUDY OF NEWER AND ESTABLISHED METHODS OF DIAGNOSING COCCIDIOIDAL MENINGITIS

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Abstract

Introduction. Meningitis is the most devastating form of coccidioidal infection. A convenient, rapid diagnostic method could result in early treatment and avoid many complications of meningitis.

Methods. CSF samples of patients with documented coccidioidal meningitis, and controls, were studied with complement fixation (CF) + immunodiffusion (ID)(considered "gold standard"), lateral flow assays (LFA; 1-strip and 2-strip), IMMY and Meridian enzyme immunoassays (EIA), beta glucan antigen (BDG), and PCR testing. The 2-strip LFA, and EIAs, enable testing for IgG and IgM antibodies separately, or aggregating the results in each test. CF with ID, or the aggregate use of IgG and IgM tests, when results available, were considered optimal use of those tests. LFAs and EIAs were evaluated at 1:21 and 1:441 dilutions of specimens. In a conservative approach, 4 observers independently read LFA assays; if any disagreements, result was scored as indeterminate. With 49 patient specimens (collected over 19 years), 40 controls, and multiple assays, we believe this the largest comparative study of CSF coccidioidal diagnostics.

Results. Results on cases and controls, using the 1:21 dilutions, are displayed:
Table. Tests in their optimal use.

	Sensitivity	Specificity	PPV	NPV
CF with ID	78%	100%	100%	74%
1-strip LFA	95%	100%	100%	80%
2-strip LFA, combined*	92%	100%	100%	91%
IMMY EIA, combined*	94%	90%	92%	92%
Meridian EIA, combined*	71%	100%	100%	68%
BDG^	96%	82%	93%	90%

Combined= assay for both IgG and IgM, either positive is scored as positive. PPV= positive predictive value. NPV= negative predictive value. Indeterminate results (≤13% in any assay) were excluded. ^from prior study

Assays of IgM separately were less sensitive. Assays at 1:441 were similarly specific but less sensitive, and suggest serial dilutions of samples could result in assays yielding titers. The agreement of positive results on cases was very good among antibody assays (87-100%), less so with BDG antigen (68-90%); also true of the correlation coefficients (r^2) comparing antibody assays only yielding positive/negative outcomes with assays (CF, BDG) that also give quantitative results. Some ventricular samples were positive in some assays. The CSF CF negative cases were of interest as likely representing specimens with the lowest antibody levels; many were positive in other assays. Our PCR is efficacious for tissue samples, but not CSF.

Conclusion. Most assays studied were efficacious in separating cases from controls. BDG antigen testing is a different methodology, and antigen-antibody complexes may explain different results.
Assays that do not require sending specimens to special reference laboratories offer logistical advantages. With the availability of kits, hospital laboratories in endemic areas can be the source of testing. LFA assays do not even require a laboratory, are simple to use, and give rapid results; potentially even at the bedside.

7

UNDERSTANDING THE ROLE OF *COCCIDIOIDES* GENOTYPE ON DISEASE OUTCOME

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Abstract

INTRODUCTION: *Coccidioides posadasii* and *C. immitis* are dimorphic fungal pathogens and the causative agents of coccidioidomycosis, commonly known as 'Valley fever'. Infection due to these fungal pathogens can range from asymptomatic to severe pulmonary disease and can disseminate throughout the body. Current studies in our laboratory demonstrate genetically distinct geographical clades of *Coccidioides*. While no studies have demonstrated a definitive difference between the pathogenesis among species, previous studies from our laboratory demonstrate potential differential immune responses among species of *Coccidioides* in murine model of coccidioidomycosis. Consequently, we **hypothesize that there is a link between *Coccidioides* genotype and overall disease outcome.**

MATERIALS AND METHODS: CD1/ICR mice were infected with $5 \times 10^5 - 1 \times 10^6$ CFU of selected *C. immitis* strains RS or 2010 or *C. posadasii* strains Silveira or C735 or given PBS for control mice. At day 4 or 5 post infection, lungs were separated into right and left lobes. The right lobe was flash frozen with N_2 and RNA extracted using a Trizol protocol. RNA was then processed for direct detection or detection with amplification for low-input samples, according to manufacturer's suggestions. The samples were then analyzed using nanoString Immunology panel or a 300-probe NanoString Custom Code Set. Statistical comparisons were implemented using nCounter software and shared vs. unique profiles determined, as well as comparison to *in vitro* samples. The left lobe was homogenized to determine cytokine/chemokine levels or CFU. Spleen and brain were also collected for CFU analysis.

RESULTS: Our results show similar pulmonary fungal burden in mice infected with either *C. immitis* or *C. posadasii* strains; however, mice infected with *C. posadasii* strains demonstrate increased dissemination in spleens and brain compared to *C. immitis* infected mice. Additionally, *C. posadasii* strain Silveira infected mice demonstrated an overall increase in the expression profile of infiltrating leukocytes compared to all other infected mice. Furthermore, we observed Silveira infected mice to have a significant increase in proinflammatory cytokines (TNFα and IL-17), anti-inflammatory cytokines (IL-4 and IL-10), and chemokine (MIP1α) compared to *C. immitis* infected mice. Lastly, the NanoString Custom Code Set demonstrated significant differences in gene expression profiles between *in vivo* and *in vitro* samples.

CONCLUSION: Thus, our studies show that the host immune response and dissemination varies among species of *Coccidioides*. Furthermore, NanoString analysis shows significant difference in transcription profile between *in vivo* and *in vitro* samples. Our studies demonstrate the potential role of *Coccidioides* genotype and association with disease variation and outcome.

8

COCCIDIOIDES POSADASII IN A DOG WITH CERVICAL DISSEMINATION COMPLICATED BY ESOPHAGEAL FISTULA

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Abstract

A 5-year-old male, neutered mixed breed dog with a history of a mass with an associated draining tract on the ventral cervical region was diagnosed with an esophageal fistula. The dog exhibited serosanguinous discharge from the draining tract, with enlarged left superficial cervical and mandibular lymph nodes, and was reported to have difficulty with deglutition of solid foods. Computed tomography revealed a communication of the draining tract with the esophagus along with enlargement of the left lateral retropharyngeal, left medial retropharyngeal, and mandibular lymph nodes. This prompted surgical exploration and debridement of the site, with closure of the esophageal fistula. Histopathology of thyroid gland, skeletal muscle, and adipose tissue obtained during surgical exploration showed spherules consistent with *Coccidioides spp.* infection. By fungal culture, PCR and DNA sequencing, *C. posadasii* was identified as the species infecting the dog. Over the course of several months of antifungal therapy, discharge from the draining tract, lymphadenomegaly, and cutaneous and subcutaneous nodules resolved. In conclusion, this is the first reported case of disseminated coccidioidomycosis to the cervical region of a dog with involvement of the thyroid gland, skeletal muscle, adipose tissue, connective tissue, and secondary esophageal fistula. *Coccidioides spp.* infections should be considered a differential diagnosis in unusual cases for dogs that live in or have traveled to endemic areas.

9

ASSOCIATIONS BETWEEN COCCIDIOIDOMYCOSIS AND DEPRESSION IN A COMMUNITY CLINIC IN THE CENTRAL VALLEY

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Abstract

Background: *Coccidioidomycosis immitis* (Valley Fever) infections are common to endemic regions, including the Central Valley in California and Arizona with 14,364 reported cases in 2017, with likely thousands more unreported cases that are either misdiagnosed or untested[1]. Valley Fever is initially introduced to its host as a pulmonary infection, but in susceptible individuals the fungus can widely disseminate and even become fatal. Although Valley Fever has treatment available, the infection is ultimately uncurable and remains a chronic condition of patients infected[2]. Other chronic diseases such as diabetes mellitus, coronary artery disease, hypertension, epilepsy, asthma, osteoarthritis and severe human immunodeficiency virus (HIV) infections have been associated with higher rates of depression[3-4]. Many chronic conditions (including Valley Fever) are associated with increased inflammation, and there is evidence that associate higher rates of depression in patients with chronic inflammatory states and with serum cytokine level disturbances[5-7]. However, severe chronic diseases are also associated with significant increase in psychosocial stressors due to increased medical necessity and impairment of daily functioning that may also contribute to the depression and inflammation itself[3,8].

Methods: In this study we analyze the results of 81 Valley Fever patients and 174 controls without valley fever, who completed the Center for Epidemiologic Studies Depression Scale (CES-D).

Results: Over 50% of the Valley Fever patients scored depressed on the CES-D, with 39% of the controls scoring depressed. This is a significant difference (p-value of 0.055) with an odds ratio of 1.56. However, patients with significant life stressors such as trouble paying bills and receiving social security disability income were significantly more likely to score as depressed on the CES-D (p-value of 0.0004).

Conclusion: For every 1.56 patients with a diagnosis of Valley Fever who are depressed, there is 1 patient that is depressed without Valley Fever. However, there is significant potential for confounding factors from this analysis as patients receiving social security disability income and patients who have trouble paying their bills more frequently scored as depressed on the CES-D compared to Valley Fever infected patients alone. Further investigation is needed to better assess the impact of psychological and social stressors due to increased medical necessity and impaired functioning from Valley Fever infections and if depression severity is correlated to disease severity.

References

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10

DISSEMINATED COCCIDIODOMYCOSIS TO THE CHEST WALL: FLESH EATING FUNGUS: A CASE REPORT

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Abstract

Introduction:
Coccidioidomycosis is an infection caused by soil-dwelling fungi, Coccidiosis's, endemic to southwestern United States, northern Mexico, and scattered areas of Latin America.1 The incidence of coccidioidomycosis has risen substantially in the past two decades: in California, the annual incidence increased from 2.5 to 8 per 100,000 between 1995 to 2006. 2 After a slight decline during 2007–09, it increased to 14 during 2010-11.3, 4 Annual rates decreased but then increased to 13.7 in 2016 with 5,372 reported cases, the highest annual number to date.5 Although pulmonary and certain extrapulmonary manifestations of coccidioidomycosis infection are well described, and typically resemble symptoms of bronchitis, pneumonia,6 and the flu,7 presentation and treatment of disseminated coccidioidomycosis of chest wall is not well studied.

Methods Used:
Retrospective study.

Summary of Results:
A 33-year-old Hispanic male presented to regional hospital with complain of chest swelling, which increased over 6 weeks and eventually became painful with mild shortness of breath. He denied having a fever, cough, night sweats, or chills but reported generalized weakness and fatigue for 6 months, which started after a cold-like illness that lasted a week. He developed painful maculo-papulovesicular rash on his upper anterior chest wall. He was evaluated by several providers prior to the ER visit and was diagnosed with dermatomal varicella zoster infection. CT scan revealed multiple loculated fluid collections in the mid portion and right side of the anterior chest wall, suggesting abscesses. These measured approximately as big as 14.5 x 9.2 x 3.2 cm. CT guided drainage was done which revealed coccidioidomycosis immitis . Extensive incision and drainage were done which left bilateral large open wound both sides of upper chest wall. He underwent several weeks of amphotericin IV with aggressive wound care which eventually allowed secondary closure. His treatment changed to high dose oral fluconazole. After a year patient's cocci titers were 1:256 with Cocci IgM and IgG reactive. After 2 more years of fluconazole his treatment was tapered and stopped by outside providers.
He presented to us with reactivation of his serology back up to 1:32 and was restarted on his treatment. During this time, he only complains of generalized weakness and is undergoing investigation of other foci of infection.

Conclusions:
A diagnosis of disseminated coccidioidomycosis should be considered in any unusual presentations in endemic areas for coccidioidomycosis.

11

CROSS-REACTIVITY OF COCCIDIOIDOMYCOSIS IN PLATELIA™ ASPERGILLUS-GALACTOMANNAN ANTIGEN - A CASE REPORT AND REVIEW OF LITERATURE.

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Abstract

Background
Coccidioidomycosis is associated with increased morbidity and mortality in solid organ transplant recipients (SOTRs). Diagnosis relies on serological tests which have lower sensitivity in the immunocompromised population. General fungal antigenic tests such as (1→3)-β-D-glucan (BDG) and Platelia™ Aspergillus Galactomannan (AGM) are used to diagnose invasive fungal infection in SOTRs, but they lack specificity. We report a case of SOTR presenting with pulmonary coccidioidomycosis, who had a positive bronchoalveolar lavage (BAL) AGM and serum BDG, with no evidence for other fungal organisms.

Case
A 70-year-old Caucasian-white female with a history of orthotopic heart transplant three years prior to her presentation. She presented with progressively worsening symptoms of fevers, headaches, cough, and shortness of breath for the last three months. She was on mycophenolate mofetil and tacrolimus medications for immunosuppression. Imaging showed multiple cavitary lung lesions. Serum Coccidioides enzyme-linked immunosorbent assay antibody Tube Precipitin (EIA-TP) and Coccidioides complement fixation (CF) was reported non-reactive and indeterminate, respectively. Serum BDG assay was elevated to > 500 pg/mL (reference range < 60 pg/mL) and serum AGM was undetectable. AGM from BAL detected positive at 2.148 (reference range < 0.5). Fungal cultures from BAL only grew *Coccidioides* species.

Discussion
On review of literature, BDG and AGM are known for their cross-reactivity in the settings of various fungal infections. A total of 85 cases in published literature report AGM cross-reactivity with *Histoplasma* (21), *Fusarium* (21), *Cryptococcus* (20), *Penicillium* (13), *Geotrichum* (3), *Blastomyces* (2), *Mycelioptho*ra (1), *Prototheca zopfii* (1), *Trichosporon* (1) *Listeria* (1) and *Nocardia* (1) species. In addition, *in vitro* studies report AGM cross-reactivity in *Blastomyces, Nigrospora, Paecilomyces, Penicillium, Trichothecium, Paracoccidioides, Histoplasma,* and *Cryptococcus* species. A laboratory-based study utilizing coccidioidomycosis patients' sera found 11 samples positive for BDG and one positive for AGM among 12 samples positive for the Miravista™ Coccidioides antigen test.

Conclusion
Antigenic tests for invasive fungal infections are non-specific and should be interpreted with caution. Physicians practicing in regions endemic for coccidioidomycosis should be aware of the cross-reactivity of BDG and possibly AGM with Coccidioides spp. However, further studies to verify this cross-reactivity are warranted.

11

CROSS-REACTIVITY OF COCCIDIOIDOMYCOSIS IN PLATELIA™ ASPERGILLUS-GALACTOMANNAN ANTIGEN - A CASE REPORT AND REVIEW OF LITERATURE.

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Abstract

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A 70-year-old Caucasian-white female with a history of orthotopic heart transplant three years prior to her presentation. She presented with progressively worsening symptoms of fevers, headaches, cough, and shortness of breath for the last three months. She was on mycophenolate mofetil and tacrolimus medications for immunosuppression. Imaging showed multiple cavitary lung lesions. Serum Coccidioides enzyme-linked immunosorbent assay antibody Tube Precipitin (EIA-TP) and Coccidioides complement fixation (CF) was reported non-reactive and indeterminate, respectively. Serum BDG assay was elevated to > 500 pg/mL (reference range < 60 pg/mL) and serum AGM was undetectable. AGM from BAL detected positive at 2.148 (reference range < 0.5). Fungal cultures from BAL only grew *Coccidioides* species.

Discussion
On review of literature, BDG and AGM are known for their cross-reactivity in the settings of various fungal infections. A total of 85 cases in published literature report AGM cross-reactivity with *Histoplasma* (21), *Fusarium* (21), *Cryptococcus* (20), *Penicillium* (13), *Geotrichum* (3), *Blastomyces* (2), *Myceliopthora* (1), *Prototheca zopfii* (1), *Trichosporon* (1) *Listeria* (1) and *Nocardia* (1) species. In addition, *in vitro* studies report AGM cross-reactivity in *Blastomyces, Nigrospora, Paecilomyces, Penicillium, Trichothecium, Paracoccidioides, Histoplasma,* and *Cryptococcus* species. A laboratory-based study utilizing coccidioidomycosis patients' sera found 11 samples positive for BDG and one positive for AGM among 12 samples positive for the Miravista™ Coccidioides antigen test.

Conclusion

Antigenic tests for invasive fungal infections are non-specific and should be interpreted with caution. Physicians practicing in regions endemic for coccidioidomycosis should be aware of the cross-reactivity of BDG and possibly AGM with Coccidioides spp. However, further studies to verify this cross-reactivity are warranted.

12

A RARE CASE OF COCCIDIOIDAL OTOMYCOSIS

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Abstract

Introduction

Coccidioidomycosis(cocci) Immitis is found abundantly in soil in the Central Valley of California, southern Arizona, west Texas, and southern New Mexico. Around 40 % of infections due to coccidioides are asymptomatic with vast majority being self-limited pulmonary infection. 1-2% of cases show dissemination beyond the lungs, most commonly to bones, ymph nodes, joints, and meninges. We here present the only case of coccidioidomycosis dissemination to the mastoid process that we experienced in our data base of over three thousand cases.

Results: Case presentation

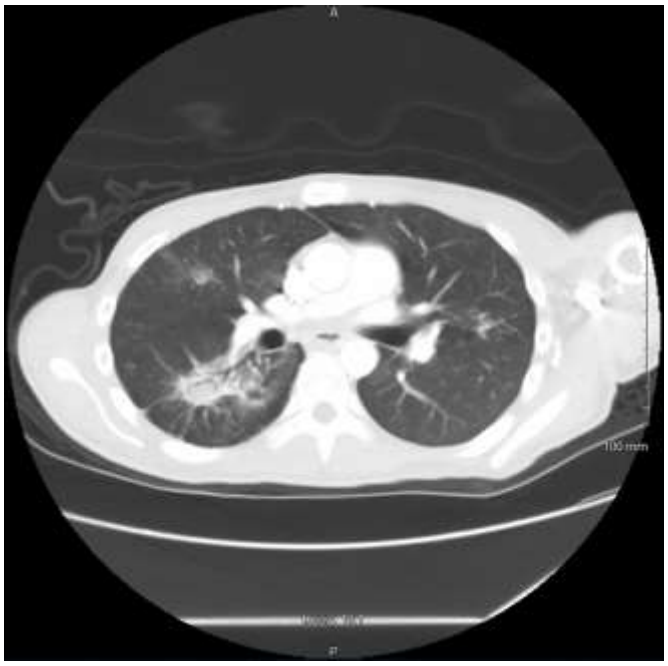
A 22 year old Hispanic male with uncontrolled Diabetes Mellitus type I was diagnosed with pulmonary coccidioidomycosis prior. Cocci complement fixing titer was 1:32. He was placed on fluconazole 800 mg daily. One year later he experienced gradual onset left ear pain with radiation to left jaw and left eye and purulent drainage. He was diagnosed with left otitis externa and was treated medically.

Subsequently, he presented with left sided progressive hearing loss and diffuse pounding headache, nausea and vomiting of 1 month duration. On otoscopic examination, tympanic membrane was erythematous, bulging with middle ear effusion. 512 tuning fork conclusive of conductive hearing loss on the left side. CT of the head showed complete opacification of mastoid air cell system on the left side with fluid in the middle ear. MRI showed left mastoiditis with an extradural collection. He was admitted and started on I.V. antibiotics.

Upon ENT consultation operative management was conducted for left mastoiditis and otitis media with left myringotomy with insertion of tympanostomy tube. Patient remained symptomatic 10 days postoperatively therefore, left mastoidectomy and tympanoplasty was performed for chronic mastoiditis. Mastoid tissue biopsy on culture grew Coccidioides Immitis after 28 days. The tympanic membrane healed well and there was a gradual increase of hearing in his left ear. Patient subsequently completed 12 week course of Liposomal amphotericin B and his treatment was changed to oral posaconazole. Patient improved and followed up in the outpatient setting.

Conclusion

Clinicians should be aware that coccidioidomycosis could hematogenously disseminate to any foci of body particularly in the immunocompromised host.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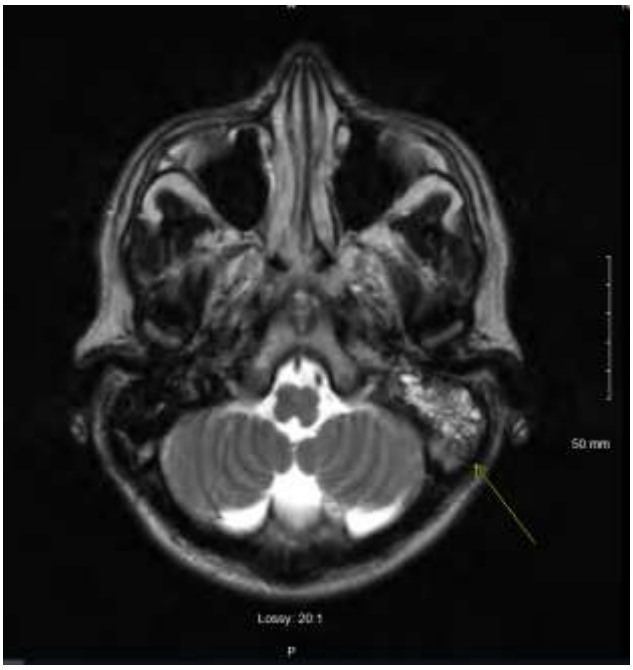
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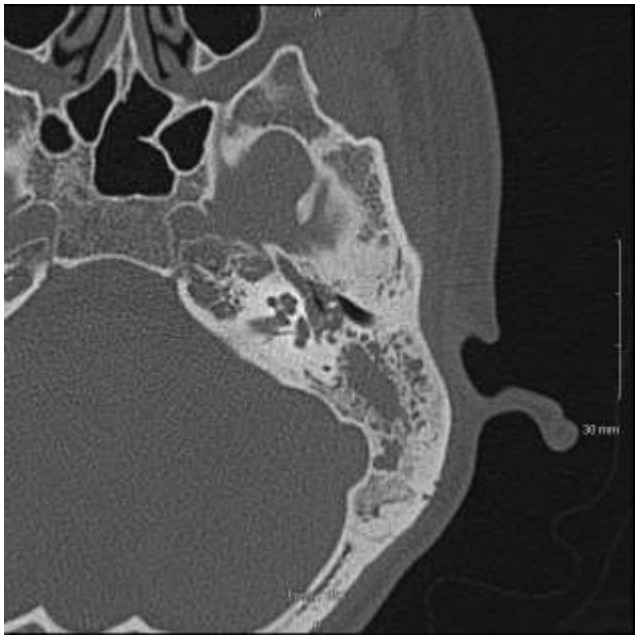
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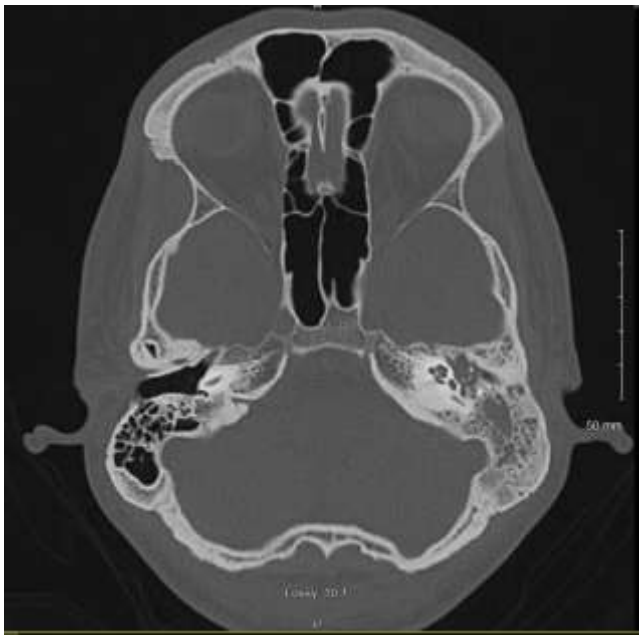
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13

TREATMENT OF FOUR REFRACTORY COCCIDIOIDOMYCOSIS CASES USING THE NOVEL ANTIFUNGAL AGENT OLOROFIM

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Abstract

INTRODUCTION: Infection due to *Coccidioides* spp. ranges from asymptomatic acquisition with resultant immunity, to severe, multifocal, life-threatening disease. There is a high failure rate with currently approved antifungals and new therapeutic options are needed. Chronic *Coccidioides* infections can lead to severe, debilitating symptoms with relatively young patients unable to perform activities of daily living or becoming dependent on oxygen or mobility aids.
Olorofim, a novel antifungal, has potent anti-*Coccidioides* activity in a murine model of infection, demonstrating the potential for sterilizing activity, as compared to fluconazole, which suppresses C*occidioides* activity in the brain but does not cure the infection.

MATERIALS AND METHODS: An open-label single-arm Phase 2b study of olorofim (ClinicalTrials.gov Identifier: NCT03583164) in patients with proven invasive fungal disease (IFD) or probable invasive aspergillosis (IA) AND refractory disease, resistance, or intolerance to available agents commenced in July 2018. Four patients with coccidioidomycosis (one each with CNS, pulmonary, paravertebral and vertebral, and multisystem disseminated disease) have to date been enrolled. All 4 patients had chronic, well-established infection not responding to therapeutic levels of azole therapy and olorofim was added in each case to the ongoing but incompletely effective background azole therapy.

RESULTS: All 4 patients demonstrated symptomatic improvements within a few weeks of adding olorofim to azole therapy with major reduction or elimination of symptoms. Example case #1: a patient who had disseminated infection (lung, CNS) but primarily with pulmonary complaints had become dependent on supplemental oxygen and use of a walking frame despite therapy with both azoles and amphotericin B over a period of 3 months. Olorofim was added to ongoing posaconazole therapy with the patient noting symptomatic improvement within three weeks. At 3 months, he no longer required the use of oxygen and was able to walk unaided. His Cocci titers also fell from 1:256 on enrolment to 1:8 after 7 months of therapy. Example case #2: a patient with chronic CNS cocci meningitis had ongoing symptoms of chronic headache, nausea, and intermittent vomiting despite 11 years of prior triazole therapy (fluconazole→ voriconazole→ isavuconazole). The patient noted symptomatic improvement within 4 weeks of adding olorofim to isavuconazole and he continues to be asymptomatic 6 months after initiation of olorofim therapy. The patient remains on combination therapy.

CONCLUSIONS: The preliminary efficacy seen to date with olorofim in patients with progressive disease (despite current therapies) suggests the potential of olorofim to address a substantial unmet medical need. F2G is currently considering potential designs for a prospective study of Olorofim as therapy for *Coccidioides.*

14

CLINICAL PRESENTATION OF SKELETAL LESIONS DUE TO COCCIDIOIDOMYCOSIS: CASE SERIES AND REVIEW OF LITERATURE.

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Abstract

Background:
Skeletal lesions (SL) due to coccidioidomycosis are a rare finding and often misdiagnosed as malignancy. We share clinical presentations, radiological features, management strategies, and outcomes of three patients diagnosed with coccidioidomycosis SL.

Case A:
A 51-year-old African American female with a history of heart transplant presented with cough, skin lesions, and right hip pain. MRI showed lytic lesions of the right iliac wing. She underwent bronchoalveolar lavage (BAL) for lung nodules that grew *Coccidioides* species. The patient was treated with fluconazole. Serial imaging showed resolution of the SL three years after treatment.

Case B:
A 25-year-old Caucasian male presented with hemoptysis, shortness of breath, and weight loss. Imaging revealed generalized lymphadenopathy and a lung cavity. A diagnosis of Hodgkin's lymphoma was made from a lymph node biopsy. PET scan revealed an expansile mass arising from the sternum with internal lytic lesions that were non-FDG avid. A biopsy of the sternal mass did not reveal evidence for malignancy. Three months into treatment, his symptoms worsened, and culture from BAL of the lung cavity grew *Coccidioides* species. After treatment with posaconazole, the sternal lytic lesions remained stable on serial imaging twelve months later.

Case C:
A 36-year-old African-American male presented with a fungating mass of his right nare. He reported multiple skin nodules, lower back pain, and twenty-pounds weight loss over a period of one month. He had diffuse lymphadenopathy suspicious for an aggressive lymphoproliferative disorder. SL was seen in the sacrum and iliac bones on MRI. A biopsy of the facial mass showed spherules. He received fluconazole and had complete resolution of symptoms one year after treatment. He did not follow up on repeat imaging.

Discussion:
Disseminated coccidioidomycosis can present with SL in 10-50% of cases. SL most often involve the axial skeleton and appear as punched-out lytic lesions on radiological imaging. In our experience, these lesions remain stable or resolve with treatment. Early diagnosis is essential to prevent disability. The choice of antifungal therapy is based upon disease extent, tolerance, and drug interactions.

15

LIVE, AVIRULENT ΔCPS1 VACCINE PARTIALLY PROTECTS IMMUNODEFICIENT STRAINS OF MICE FROM LETHAL COCCIDIOIDES INFECTION

Lisa Shubitz[1], Daniel Powell[2], Amy Hsu[3], Christine Butkiewicz[1], Hilary Moale[4], Hien Trinh[1], Thomas Doetschman[5], Lourdes Lewis[1], Steven Holland[3], Jeffrey Frelinger[1], John Galgiani[1]

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Abstract

Introduction

Inborn immunodeficiencies, such as IL-12 deficiency, hyper IgE syndrome, and STAT1 and STAT4 mutations, as well as therapeutic immune modulation, e.g., TNF inhibition, have been reported to cause severe to fatal coccidioidomycosis. Mitigation of the severity of coccidioidomycosis in immunodeficient patients through vaccination has the potential to reduce morbidity and mortality among vulnerable populations. We have shown protection by *Δcps1*, a live, avirulent coccidioidal vaccine, by reduced lung fungal burden, prevention of dissemination, and prolongation of survival in wild type mice. These studies explore efficacy of the vaccine to provide protection in mice with single-gene immunodeficiencies that have been documented to have a negative impact on coccidioidomycosis in humans or in mice.

Materials and Methods

In a series of studies, C57BL/6J (B6), B6D2F1/J (B6D2F1), B6.129S7-*Ifngr1*^{tm1Agt}/J (IFNγr1 KO), C57BL/6-Tg(Stat3*)9199Alau/J (STAT3 KO -hyper IgE), B6.129S6-*Clec7a*^{tm1Gdb}/J (Dectin-1 KO), C57BL/6J-*Stat4*^{em3Adiuj}/J (STAT4 KO), and C57BL/6N-*Stat4*^{E626G} (STAT4 E626G) mutant mice were vaccinated twice subcutaneously with 10,000 viable *Δcps1* spores and challenged intranasally 4 weeks later with 50 or 100 spores of *C. posadasii*, strain Silveira. Studies were sacrificed at 14 days p.i. with enumeration of lung and spleen fungal burdens.

Results

Vaccination with *Δcps1* significantly reduced lung fungal burdens compared to unvaccinated B6, B6D2F1, or immunodeficient control mice for Dectin-1 KO (P=0.02), IFNγr1 KO (P=0.036), STAT3 KO (P=0.03), STAT4- KO (P<0.001), and STAT4^{E626G} X DBA2/J (P=0.001). Splenic dissemination was detected in fewer vaccinated than unvaccinated immunodeficient mice with infectious doses of 50 spores, but prevention of dissemination was less evident with 100 spore inoculations. However, spleen fungal burden was significantly reduced in all strains (P<0.01).

Conclusions

Preliminary investigation shows that vaccination with the live, avirulent vaccine, Δcps1, can overcome single-gene immunodeficiencies and reduce the impact of coccidioidomycosis on this susceptible population. We theorize that the array of antigens in the whole-organism vaccine allows the immune system to utilize alternative pathways for antigen presentation that produce protective immunity.

16

A MODEL FOR CHRONIC COCCIDIOIDAL INFECTION IN MICE USING *COCCIDIOIDES POSADASII*, STRAIN 1038

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Abstract

Introduction

Virulent strains of *Coccidioides posadasii*, such as Silveira, and *C. immitis*, such as RS, given intranasally generally cause death in mice within 2-3 weeks post-infection. A more slowly progressive or self-arresting infection model would improve the study of innate and adaptive immune responses. BALB/c mice were reported to survive intranasal infection with *C. posadasii*, strain 1038. We have expanded this observation to develop a more chronic model of progressive infection and another of stable fungal burden similar to what occurs in many humans.

Materials and Methods

C57BL/6 (B6), B6.129S7-*Ifngr1*^{tm1Agt}/J (IFNγr1 KO), B6.129S-*Tnf*^{tm1Gkl}/J (TNFα KO), and B6D2F1 mice were infected with ~50 spores of strain 1038 or Silveira intranasally. In some studies, mice were sacrificed at pre-specified time points for quantitative cultures of lungs and spleens. In others, survival was monitored for up to 10 weeks and spleen cultures were assessed qualitatively.

Results

Mean lung fungal burden (LFB) at 14 days p.i. in both B6 and IFNγr1 KO mice was ~10^7 for Silveira and lethal, but only ~10^4 for 1038. With 1038 infection, IFNγr1 KO mice survived a median of 43.8 days compared to 70 days in B6 ($p=0.005$). Separately, TNFα KO mice survived only 22.5 days compared to 69 days for B6. With B6D2F1 mice monitored for 10 weeks following 1038 infection, 10/10 animals survived. LFBs of B6D2F1 mice (n=5/group) enumerated at 4, 8, and 16 weeks had mean LFB of $10^{4.5}$, 10^3, and 10^4, respectively. Though mice gained weight throughout, 2/5, 4/5 and 4/5 mice had splenic dissemination at 4, 8, and 16 weeks, respectively (fungal burden range, 0-3800 cfu).

Conclusions

Strain 1038 produces a chronic coccidioidal infection in mice that robustly reveals reduced host resistance where immunologically important genes are missing, an effect poorly demonstrable in infections produced by Silveira here or by RS in studies by others. Further, 1038 infection of B6D2F1 mice produces a protracted, stable fungal burden that may recapitulate arrested primary infections seen in humans. These models should now permit studies not previously possible, such as of the impact of biological response modifying drugs on immune control of coccidioidal infection.

17

CAVITARY LESIONS IN A DOG WITH SEVERE, CHRONIC PULMONARY COCCIDIOIDOMYCOSIS

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Abstract

Introduction
Cavitary lung lesions due to coccidioidomycosis are extremely unusual in canine coccidioidomycosis. The dog of this report had multiple cavitary lesions observed on pulmonary CT.

Materials and Methods
A 4 YO SF German wirehaired pointer dog was initially presented for severe cough, wheezing, lethargy and weight loss; the dog was diagnosed with coccidioidomycosis based on serology (IgM+, IgG+, titer 1:16), radiography, clinical and clinicopathologic findings, and travel history. The dog was subsequently treated with fluconazole with minimal clinical or radiographic improvement over several months. In the three months preceding referral, she was treated sequentially with 6 weeks of posaconazole and 7 infusions of amphotericin B lipid complex, which was not tolerated, then itraconazole and voriconazole briefly. At referral presentation, she was thin with a harsh cough and tachypnea, neutrophilia, monocytosis, and mild nonregenerative anemia. The dog's thorax was evaluated by computed tomography, revealing severe tracheobronchial lymphadenomegaly obstructing mainstem bronchi and occluding the right middle bronchus. There was a ground glass appearance to the right middle lung lobe, along with a diffuse bronchointerstitial pattern and 30-35 nodules (5-26 mm) thoughout all fields. Five cavitating lesions (1.4-2.8 cm) with communication to branching bronchi were also identified.

Results
The dog's treatment was changed back to posaconazole, 5 mg/kg/day with a meal, and prednisone 0.5 mg/kg daily. At 1-month recheck, the dog was clinically improved with weight gain and increased energy. The posaconazole was well-tolerated. Interim blood tests at 3-4 month intervals showed a slow decrease in neutrophilia and monocytosis over time and the RBC count returned to normal. At 1-year recheck, the dog remains on a low every other day dose of prednisone, is active, a good body weight, and rarely coughs. Repeat CT shows reduced compression of mainstem bronchi and opacity of right middle lung lobe. Nodules are slightly smaller and some are mineralized. The cavitated lesions are still present though mildly reduced in size.

Conclusions
Though rare, cavitary lesions can occur in dogs with coccidioidomycosis. Radiographic improvement is lagging clinical improvement in this dog. CT imaging greatly improved delineation, treatment decisions, and follow up of extensive thoracic pathology in this dog.

18

A NATURAL HISTORY STUDY OF IMPORTED VALLEY FEVER IN THE DAKOTAS

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Abstract

Introduction
Less than 2% of US valley fever cases are reported outside the endemic states of Arizona, California, Nevada, New Mexico, and Utah, but there is continued flow of people between the highly endemic areas and other parts of the US. Physicians in non-endemic areas may be unfamiliar with valley fever, raising concerns for delayed diagnosis and poor outcomes.

Materials and Methods
A natural history study of cases of valley fever was initiated at our large Upper Midwest health system in February 2015. Cases are identified through referrals to the investigator's clinic from pulmonology, infectious disease, and primary care practices. With the patient's consent, data are collected retrospectively from medical records and prospectively from medical records and at clinic visits.

Results
As of January 2020, 10 patients have been enrolled, and data are available for 9. For comparison, during 2015-2019, 20 total cases were officially recorded by the South Dakota Department of Health, and 28 by the North Dakota Department of Health. All cases were acquired in Arizona, but only 5/9 were diagnosed there. The remainder were diagnosed locally by serology (3) or culture (1). 7/9 cases were pulmonary and 2/9 were disseminated. 4/9 patients had some form of immunosuppression. All patients were treated with azole antifungal agents. As of January 2020, 1 was deceased from other causes, 1 remained on fluconazole for a history of severe pulmonary infection, 4 improved and were discharged from the infectious disease clinic, and 3 remained in care but were stable off antifungal therapy.

Conclusion
Clinicians in the Dakotas are seeing valley fever patients, including some who are not diagnosed until they have left the endemic area. This small sample shows a mixture of pulmonary and disseminated cases, with and without immunosuppression. In this group no severe outcomes have been observed.

19

Identification of Spherule Antigens from Infected Lung Tissue Using Laser Capture Microdissection

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Abstract

We used Laser capture microdissection (LCM) coupled with mass spectrometry to identify Coccidioidal biomarkers from infected lung tissues. This technique takes a "snapshot" of proteins produced *in vivo* during *Coccidioides spp.* infection in human lungs. Proteomic analysis of LCM lung sections revealed hundreds of host and Coccidioidal proteins. Twenty-seven *Coccidioides spp.* proteins were identified which do not share significant sequence orthology with human proteins. Three of the 27 Coccidioidal proteins are potential *Coccidoides*-specific biomarkers, as they also do not share sequence homology to any other pathogenic fungus or microbe. Gene ontology analysis of the 27 biomarker candidate proteins revealed enriched hydrolase activity and increased purine and carbohydrate metabolism functions. Finally, we provide proteomic evidence that all 27 biomarker candidates are produced by the fungus when grown *in vitro* in a media- and growth-phase dependent manner and conclude that six Coccidioidal proteins were significantly more highly abundant in spherules growing in lung tissue than in laboratory-grown spherules or mycelia.

20

THE PHARMACOKINETICS OF FLUCONAZOLE IN ALPACAS

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Abstract

INTRODUCTION

Reports indicate that alpacas develop severe, wide spread, disseminated coccidioidomycosis and treatment is often ineffective. Alpacas are functional ruminants with a large fermentation chamber orad to the glandular stomach and small intestine. Bioavailability of oral medications is reduced or negligible compared to monogastric species. This study aims to determine the dose of orally administered fluconazole necessary to develop blood levels expected to be therapeutic for coccidioidomycosis and other susceptible fungal infections.

MATERIALS AND METHODS

Client-owned alpacas were screened with CBC/serum chemistries and enrolled with owner consent. Pairs of animals were treated with fluconazole every 24 hrs for 13 days to achieve steady state. On day 14, an anticoagulated blood sample was obtained prior to final fluconazole dose, and 1-3, 6-8, and ~24 hours post-dose. Plasma was separated and frozen at -80°C until analysis. Fluconazole levels were determined by HPLC-mass spectroscopy. 24-hr AUC was determined and a population PK model constructed to calculate a therapeutic dose. Bioavailability was estimated from results.

RESULTS

The starting dose of 6 mg/kg yielded 24 hr AUCs of 100 and 200 mg h/L. Fifteen mg/kg in the next two animals resulted in a 24 hr AUC over 1000 mg h/L. With a target 24-hr AUC of 400-800 mg h/L, a dose of 10 mg/kg was predicted to yield AUCs in the therapeutic range. One animal was in this range and the second was below this around 300 mg h/L. Three additional animals were administered 10 mg/kg to verify the PK model, but all were low. Bioavailability of the 6 mg/kg dose was calculated at 50% and of the 15 mg/kg dose near 100%. 4/9 animals had unpredicted low 24-hr AUCs at 10 mg/kg and bioavailability of ~30% on the day of 24-hr blood sampling.

CONCLUSIONS

Therapeutic plasma levels of orally administered fluconazole can be achieved in alpacas with daily doses of 10-12 mg/kg, which were well-tolerated. The wide variation in bioavailability makes therapeutic drug monitoring appropriate for animals with poor responses. Bioavailability may be affected by differences in environment, husbandry, age, or physiology of animals and is a topic for further investigation.

21

A Case of Pediatric Coccidioidomycosis Meningitis

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Abstract

Coccidioidomycosis is a common fungal infection usually seen in the South-Western areas of North America. Though the infection presents with flu like symptoms, complications can arise such as pneumonia or meningitis. Pediatric complication of coccidioidomycosis are rare and as a result are not frequently studied.
The case presented is of a 7-year-old male with no history of coccidioidomycosis who presented with pneumonia like symptoms. Patients arrived a few days later with meningeal symptoms. Though prophylactic treatment was given for meningitis, patient continued to have fevers and did not improve resulting in a grand-mal seizure lasting 1 minute and 30 seconds. Patient was treated with Fluconazole and Keppra. An MRI with contrast was done showing a small presumed lacunar infarct in the left caudate head of the inferior medial left frontal lobe. An EEG conducted during his stay indicated mild encephalopathy. However, a cognitive test indicated normal age appropriate behavior and mentation.
A lumbar puncture was performed on admission and indicated a negative CSF IgG with insufficient sample size for IgM. Serum IgG titers showed a 1:4 ratio with insufficient sample size for IgM analysis. All other causes for meningitis such as HSV and Enterovirus were negative.
In the course of a coccidioidomycosis infection, 90% of patients have IgM antibodies in their serum by three weeks whereas only less than 50% have IgM present a week after symptom onset. As a result, false negative readings are common in the early stage of coccidioidomycosis infection. As a result, retesting via serum and CSF are usually more sensitive following 2-3 weeks after symptoms onset [(3)]. In this case, patient was noted to have positive IgG antibodies in their serum but none in their CSF. As a result, following up with a repeat lumbar puncture is crucial to confirm the etiology of his meningitis. Very few cases have been reported in the pediatric population related to meningitis as a complication of coccidioidomycosis infection. As a result, establishing a standard of care when encountering such cases is imperative.

22

COMPARISON OF A NOVEL RAPID LATERAL FLOW ASSAY (LFA) TO ENZYME IMMUNO ASSAY (EIA) RESULTS FOR EARLY DIAGNOSIS OF COCCIDIOIDOMYCOSIS

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Abstract

Introduction. Prompt diagnosis and appropriate management of coccidioidomycosis (Valley fever, VF) depends on laboratory testing, most frequently by EIA where results take days to become available. An FDA-cleared LFA [sōna, IMMY] could reduce reporting time to less than an hour. In a prospective observational study at our institutions, we compared this LFA with a standard EIA [IMMY] performed commercially.

Methods. From 1/2/2019 to 12/31/2019, patients for whom emergency or hospital clinicians ordered EIAs for necessary care were invited to be studied unless they had a previous VF diagnosis or were < 18 years old. Consenting subjects completed a questionnaire, permitted access to their medical records, and provided blood for LFA [if LFA^+, read as 1^+- 4^+], erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and procalcitonin (PCT) tests. All tests were performed in CLIA-regulated laboratories.

Results. Of 401 enrolled subjects, the average age was 56 years, 52% were female, and 74% were inpatients. Of 59 EIA^+ subjects, 18 EIA^+ were IgM^+ only but 10 of these had other confirmatory laboratory evidence of VF. Indeterminants [4 sera with LFA^+] were treated as negative. LFA sensitivity was 29% [95% Confidence Interval (CI) =18-42%], specificity was 92% [CI=88-95%], PPV was 40% [CI=28-54%] and NPV was 87% [CI=85-90%]. The median duration of illness in LFA^+/EIA^+ subjects [n=17] was 21 days; LFA^-/EIA^+ was 14 days [n=42] [p=0.10]. The LFA^+ intensities were lower in LFA^+/EIA^- sera [n=25] compared to intensities in LFA^+/EIA^+ sera (p=0.003). The clinical presentations of all groups were similar. For EIA^+, ESR were elevated [mm/h>21 for males, >31 for females] in 31 [53%], CRP were >8 mg/L in 41 [70%]. PCT results were <0.25 ng/ml [bacterial infection unlikely] in 46 [78 %] that were EIA^+. Antibacterial drugs were prescribed for 48 [81%] of patients with PCT<0.25 ng/ml.

Conclusion. In early diagnosis of VF, this LFA test had a specificity of 92% but lower sensitivity relative to EIA results. LFA^-/EIA^+ subjects trended with shorter illnesses than LFA^+/EIA^+ subjects, and LFA^+ intensities were lower when EIA was negative. Additionally, antibacterial therapy might have been avoided in 78% of EIA^+ subjects had PCT results been used.

23

COCCIWATCH: RESULTS OF 6-MONTH AIR-SURVEILLANCE IN ARIZONA

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Abstract

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

MATERIALS AND METHODS
For this project, air-samples were collected daily at 11 locations around Phoenix, AZ and at three locations in Kern County, CA. DNA is extracted and tested for the presence of *Coccidioides* DNA using previously developed qPCR assays.

RESULTS
We will present preliminary testing results from filters collected 1/6/2018 and 7/6/2018 from Arizona, which provide evidence of seasonality and uneven spatial distribution of *Coccidioides* in the air.

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

24

VALLEY FEVER IN ARIZONA: SEARCHING FOR THE DUST DEVIL

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Abstract

INTRODUCTION
Coccidioides immitis and *C. posadasii* are endemic fungal pathogens distributed throughout arid and semi-arid regions of both American continents, and are the causative agents of coccidioidomycosis. The state of Arizona has the highest overall incidence of disease in the United States. Genetic analysis to date suggests that the majority of clinical isolates recovered from Arizona belong to *C. posadasii* species. However, little is known about the prevalence species distribution and ecological factors that could favor the occurrence of this pathogen in the environment. Previous efforts to understand and map the ecological niche of *Coccidioides* have had limited success. Applying molecular techniques to identify the fungus in the soil is a breakthrough that allows for large scale mapping of the organism in the environment, providing data for geospatial and temporal mapping of the pathogen. Solving the question of where and when the organism is at highest prevalence will help to protect the health of Arizonans.

MATERIALS AND METHODS
Recent work from our group has shown that the real-time qPCR technique is successful at detecting soils positive for *Coccidioides*. We anticipate that mapping the prevalence of other organisms in the *Coccidioides* positive and negative soils will improve our understanding of the ecological niche of this vastly understudied fungal pathogen. Soil, dust, and wildlife tissue samples were collected in suspected endemic regions. DNA extractions were performed, and real time PCR-based approaches and amplicon sequencing were used to detect *Coccidioides* DNA from these varied samples.

RESULTS
Variation in the amount of *Coccidioides* DNA was observed depending on the sample type and origin. Surprisingly, highly positive samples were found in all regions in Arizona. The analysis of positive soils revealed a close association with animal burrows. These results indicate that *C. posadasii* is endemic throughout Arizona, but varied amounts of *Coccidioides* DNA in the soil may result in different amounts of fungal inoculum, and consequently differential exposure to humans. Additionally, there may be different genotypes present in the environment and in wildlife, which may have differential infection and disease dynamics.

CONCLUSION
Previous efforts to understand and map the ecological niche of *Coccidioides* have had limited success. Solving the question of where and when the organism is at highest prevalence will help to protect the health of Arizonans, visitors, and potentially all residents of endemic areas to improve prevention in high risk population groups.

25

TRANSCRIPTOMICS OF THE COCCIDIOIDOMYCOSIS CANINE VACCINE REVEALS INSIGHTS INTO THE FUNCTION OF CPS1

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Abstract

INTRODUCTION

The coccidioidomycosis canine vaccine currently being developed is a *Coccidioides posadasii* Silveira mutant with a whole gene deletion of *CPS1*. The virulent Silveira and avirulent *Dcps1* strains differ in their ability to produce spherules *in vitro*: Unlike Silveira that produces enlarged spherules that release endospores, *Dcps1* spherules readily undergo plasmolysis without differentiating into endospores. Sequence analysis of *CPS1* suggests it encodes a membrane receptor protein that regulates downstream gene expression, but which genes it regulates are unknown. To gain insights into the function of Cps1, we compared the transcriptomes of Silveira and *Dcps1* during mycelial and spherule growth *in vitro*.

MATERIALS AND METHODS

To perform RNA-seq, RNA was harvested from 24 h and 48 h spherules, and 48 h mycelia of Silveira and *Dcps1*. Spherules were grown in RPMI with 20% CO_2 and mycelia were grown in 2xGYE liquid media, both at 37 °C. Three biological replicates were prepared for each condition. Illumina reads were mapped to the *C. immitis* RS genome and analyzed for differentially expressed genes.

RESULTS

RNA-seq analyses revealed significant differences in gene expression between *Dcps1* and Silveira. In 24 h *Dcps1* spherules, 226 genes were up-regulated and 77 were down-regulated; in 48 h *Dcps1* spherules, 144 genes were up-regulated and 258 were down-regulated; in 48 h *Dcps1* mycelia, 558 genes were up-regulated and 469 were down-regulated. Several genes that have been demonstrated to play a role in virulence in *Coccidioides* and other human pathogenic fungi were found amongst the differentially expressed genes. Enrichment analyses revealed overrepresentation of genes encoding components of the membrane and those involved in oxidoreductase activity and amino acid metabolism.

CONCLUSION

The large number of differentially expressed genes across multiple pathways between *Dcps1* and Silveira supports the proposed role of Cps1 as a regulatory protein and provides a platform for identifying processes that are defective in the mutant especially during spherulation. Genes with the same expression pattern as *CPS1* across different conditions may indicate co-regulated genes and serve as additional targets for vaccine development. Understanding the biological consequences of the *CPS1* deletion is key to demonstrating the safety of this live-attenuated vaccine.

26

TRANSLATION OF RESEARCH INTO A LICENSED VETERINARY VACCINE: BACK PASSAGE STUDIES FOR AVIRULENT ΔCPS1 COCCIDIOIDES POSADASII SPORE CANDIDATE VACCINE

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Abstract

INTRODUCTION
Title 9, Code of Federal Regulations, (102.5/ 104.5) provides recommendations about the design/conduct of backpassage studies to support applications for a Veterinary Biological License. Attenuated vaccines must be tested to assure that the strain does not revert to virulence (RtV). This requires that the vaccine microorganism be evaluated to ensure an acceptable margin of safety.

Backpassage studies consist of successively propagating vaccine Master Seed (MS) through a series of backpassages in vivo. Study designs administer the MS microorganism to susceptible host animals, and, after an appropriate incubation, one recovers the microorganism and administers it to a second group of susceptible host animals. The CFR stipulates a minimum of five successive passages in 10 animals with no in vitro propagation permitted. Subjects should be the most susceptible age, seronegative (SN) and exposed above the target release potency by the route most likely to lead to replication and RtV.

METHODS
Mouse Pilot- On SD 0, 20 C57BL/6 mice/group were intranasally inoculated with either 10K or 30K Δcps1 pre-Master Seed (pMS) spores and 5 mice from each group were sacrificed SD7, SD14, SD21 or SD28. Lung tissue was homogenized and plated to quantitate fungal burden. 500 µl from each homogenate was pooled, strained to remove gross debris, and centrifuged to adjust volume to 400 µL. 30 µL was reinoculated IN to 10 new mice which were sacrificed per cohort 14 or 28 days later to quantify fungal burden.

Dog Pilot- Three SN 6-month-old dogs were inoculated intratracheally with 100K pMS Δcps1 spores and monitored for 28 days.

RESULTS
From the initial IN exposure, fungal growth was detected in 3/20 mice inoculated at 10K and 4/20 at 30K. In the 2nd passage, there was no fungal growth. For the dogs, clinical signs, hematology, thoracic radiography, and serum chemistries remained normal. At necropsy on SD28 there were no gross lesions and no fungal growth in lungs or mediastinal lymph nodes.

CONCLUSIONS
These pilot studies provide strong evidence that the Δcps1 vaccine, as tested, does not show any RtV and provides a strong structural basis to discuss options to the codified requirements.

27

DISSEMINATED COCCIDIODOMYCOSIS PRESENTING AS POLYARTICULAR SEPTIC ARTHRITS: A CASE REPORT

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Abstract

INTRODUCTION:
Coccidioidomycosis is a fungal infection endemic to southwestern United States. It is caused by inhalation of spores of Coccidioides. 60% of infections are asymptomatic; the remaining 40% are primarily pulmonary disease. In less than 1% of infections, dissemination can occur. Dissemination usually affects those with impaired cellular immunity, and can involve bones, joints, meninges, and skin [1]. We present a rare case of disseminated polyarticular coccidioidomycosis that presented with three isolated septic joints.

MATERIALS AND METHODS:
Retrospective case report.

RESULTS:
We present the case of a 29-year-old Hispanic male who presented to the emergency department (ED) complaining of pain and swelling of right wrist, right ankle, and left knee for three months. Previously, he was evaluated in the ED and outpatient rheumatology for left knee pain with workup suggestive of reactive arthritis secondary to chlamydial infection which he was treated for. However, he returned to the ED due to the involvement of two more joints as-well-as a new onset of spontaneous purulent drainage from his wrist. In the ED, an arthrocentesis of two of the joints showed total nucleated cells of 520,000/cm^2 and 90,000/cm^2 with 61% and 93% Neutrophils respectively. Fungal culture eventually grew *Coccidioidomycosis Immitis* from his wrist and knee. Coccidioidomycosis Complement fixation titer came back > 1:512. Bone scan showed uptake of adjacent bones in the affected joints. Liposomal amphotericin B was started with slow improvement. Antifungal susceptibility testing for Amphotericin B, Natamycin, Fluconazole, Itraconazole, Posaconazole, Voriconazole, and Isavuconazole were 0.06, 4, 4, 0.25, 0.125, 0.25, and 0.5 mcg/mL respectively. Due to continuous drainage he developed superimposed bacterial infections of his right wrist and left knee and was treated with antibiotics along with the IV Amphotericin. He underwent several therapeutic arthrocentesis of the knee and eventually was switched from IV Amphotericin to prolonged oral Isavuconazonium therapy with close outpatient follow up.

CONCLUSION:
Clinicians should keep the diagnosis of disseminated synovial coccidioidomycosis in mind in the endemic area. However, disseminated polyarthritis coccidioidomycosis has not been seen in our experience. The distinction from other etiologies mostly rheumatological causes will remain challenging.

28

DISSEMINATED COCCIDIOIDOMYCOSIS IN A PATIENT WITH HIDRADENITIS SUPPURATIVA.

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Abstract

Background
Hidradenitis Suppurativa (HS) is a chronic inflammatory skin disease with a prevalence of 1 - 4 percent. The co-occurrence of HS with coccidioidomycosis has not been described in literature. In this case report, we present a patient with known HS and recurrent super-infection on the left anterior chest wall. Left axillary lymph node biopsy demonstrated the presence of *Coccidioides* spherules.

Case
41-year-old male, originally from Pacific Islands, with past medical history of uncontrolled diabetes, chronic kidney disease and HS presented with increased pain, swelling and discharge of a chronic left chest wall lesion. He also reported coughing and worsening shortness of breath over the past several weeks. Computed tomography (CT) imaging of chest revealed severe left chest wall inflammatory changes, subcutaneous fistula and left axillary lymphadenopathy. CT lung findings showed multi-focal nodular lesions, mediastinal lymphadenopathy and a 4.2 cm mass like consolidation in the left lower lobe. Chest wall wound bacterial cultures grew Streptococcus constellatus, Proteus mirabilis and anaerobes. The left axillary lymph node biopsy revealed necrotizing granulomas with spherules seen on Grocott's Methenamine Silver (GMS) staining and the fungal cultures grew *Coccidioides* species.

Serum Coccidioides enzyme-linked immunosorbent assay (EIA) was reactive. Initial serum Coccidioides complement fixation (CF) titer was negative. Repeated CF titer one week later was > 1:256. Screening tests for HIV, Syphilis, Hepatitis B, Hepatitis C and autoimmune panel were negative. Total serum IgG was 2700. Patient was immediately started on treatment with fluconazole. Follow up CF titer obtained 2 months later was 1:64 with resolution of respiratory symptoms and stable appearance of lesion on the left chest wall.

Discussion
HS has been correlated to certain immunologic, genetic and environmental factors. Observational studies report a significant correlation with inflammatory bowel disease, metabolic syndrome, arthritis, and depression. To our knowledge this is the first described case of disseminated coccidioidomycosis in patients with HS.

29

A RECOMBINANT CHIMERIC POLYPEPTIDE VACCINE (RCPA1) CONFERS PROTECTION FOR HLA-DR4 TRANSGENIC MICE AGAINST BOTH COCCIDIOIDES POSADASII AND COCCIDIOIDES IMMITIS

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Abstract

INTRODUCTION: Coccidioidomycosis is a fungal infection caused by inhalation of *Coccidioides posadasii* (*Cp*) or *Coccidioides immitis* (*Ci*). The two species are phenotypically similar, but they show highly differentiated genomes and differences in their geographic distribution. We have created a rCpa1 peptide derived from *Cp* C735 isolate that contains 3 antigens (Ag2/Pra, Csa and Pmp) and 5 human MHC II-binding peptides that can activate protective CD4 T-cell response. Immunization with rCpa1 combined with glucan-chitin particles (GCP-rCpa1) confers protection for both C57BL/6 and HLA-DR4 transgenic mice against the autologous isolate. The goals of this study are to analyze amino acid (aa) sequences of the rCpa1 among *Cp* and *Ci* isolates and to evaluate cross-protection and immune response of the GCP-rCpa1-vaccinated mice against *Ci*.

MATERIALS AND METHODS : The deduced aa sequences of 39 and 17 clinical isolates of *Cp* and *Ci*, respectively, were aligned to the rCpa1 sequence. Mice were subcutaneously vaccinated 3 times with 200 µg GCP loaded with 10 µg rCpa1 at 2-week intervals and intranasally challenged with a potentially lethal dose of spores at 4-week after the final vaccination. Fungal burden (CFUs) were determined at 14 dpc. Pulmonary T cells were analyzed using intracellular cytokine staining methods.

RESULTS: The aa sequences of rCpa1 are identical among isolates of the same species, while there are 7 aa substitutions between *Cp* and *Ci*. Six of the seven altered aa residues are not located on the predicted human MHC II binding sites. Both vaccinated C57BL/6 and HLA-DR4 mice had significantly reduced fungal burdens in their lungs and spleens after an intranasal challenge with one of 3 *Cp* and 2 *Ci* isolates compared to corresponding nonvaccinated mice. T-cell assays revealed that the GCP-rCpa1 vaccine elicited a mixed Th1 and Th17 response in the lungs of vaccinated mice against *Ci*, comparable to the immune response against *Cp*.

CONCLUSION: These results suggest that the GCP-rCpa1 vaccine confers cross-protection against both *Cp* and *Ci* by activating a mixed Th1 and Th17 response. Currently, we are applying bioinformatics and T-cell assays to identify additional MHC-II binding peptides for refining the design of a board-spectrum vaccine against coccidioidomycosis.

31

MYCOSES STUDY GROUP SCORE 2020: A SYSTEM FOR EVALUATING THERAPEUTIC RESPONSE IN THE NEW MILLENNIUM

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Abstract

Background

Four decades ago, *Dismukes et. al* developed the Mycoses Study Group (MSG) score to evaluate the efficacy of treatment in a broad array of fungal infections. Subsequently, the score was modified to evaluate specific infections, most particularly Coccidioidomycosis. This scoring system has served well for forty years. However, our group wanted to develop improvements in this scoring system that eventuated in a more objective score. The goal was to decrease reliance on symptoms and radiographic results as they are subject to variation and interpretation. We decided to increase the role of numeric laboratory values in the score system to obtain this a more objective view of progress throughout therapeutic intervention.

Methods

We reviewed the original *Dismukes et. al* paper and multiple iterations of the scoring system as used in coccidioidal therapeutic trials. It was decided to revise both the non-meningeal and meningeal score systems.

Results

The original non-meningeal score system, as used by *Catanzaro et. al,* was weighted for pulmonary disease. The revised non-meningeal MSG 2020 is more applicable for severe pulmonary and disseminated disease to any site.

Included in the revised non-meningeal score system are weight loss, eosinophilia, markers of inflammation and skin tests for all patients. There is a separate section for pulmonary disease with physiologic scoring for severity. Specific sections for skin, subcutaneous abscess, joints, bone, intraabdominal, lymph nodes, and other disseminated sites are listed.

The revisions to the MSG score for meningeal disease was revised to simplify and objectify the analysis of symptoms. The evaluation of mental status was modernized. A new section for increased intracranial pressure was added to include this critical advance in knowledge about initial and subsequent patient care.

Conclusion

The goal of this effort is to modify the original and its successors to be more objective and to incorporate a newer understanding of coccidioidal disease.

As stated by *Dismukes et. al*

"We hope the spirit of these remarks will spark lively discussion as well as constructive criticism, challenge, and controversy … if indeed such healthy discussion, argument, and dialogue ensues, then we will have satisfactorily accomplished our goal."

32

CLINICIAN PRACTICE PATTERNS THAT RESULT IN THE DIAGNOSIS OF COCCIDIOIDOMYCOSIS BEFORE OR DURING HOSPITALIZATION

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Abstract

Introduction: Because we found diagnostic delays for coccidioidomycosis (Valley fever or VF) of >1 month for 43% of patients in our Arizona primary care units, we initiated a training program. In this report, we describe practice patterns that existed in the two years immediately prior to and the first year of the program's implementation.

Methods: We queried Banner Health electronic health and billing records for adult patients during 2017, 2018, and 2019 in 15 hospitals, 53 primary care clinics and 48 urgent care sites in Arizona. As previously studied, we considered the ICD10 diagnosis code for VF valid if a positive serologic result was returned within 30 days before or 60 days afterward. We programmatically identified related health care encounters by searching six months prior to VF diagnosis for a "coccidioidal syndromic symptom" (CSS).

Results: For these 3 years, 2,043 VF cases occurred, 72.9% during hospitalization, 13.6% in primary care clinics, 8.1% in specialty clinics, 3.2% in emergency departments, and 0.5% in urgent care clinics. In a subset of hospital diagnoses, 750 (50.3%) needed neither ICU nor hospital-requiring procedures, had a median length of stay <3 days, and had total hospital charges of $69 million (median $50,757 per patient). Charges for all diagnostic hospitalizations were $167 million. Visits with CCSs prior to hospital diagnoses occurred in 673 (45% of hospital diagnoses), during which VF tests were only done in 29%. For patients with assigned primary care physicians, 74% had a CCS in a previous visit (ambulatory or hospitalization) before the diagnostic hospitalization. Primary care clinicians increased their annual frequency of ordering VF serology from 6.6 to 7.5 ($p<0.001$, Wilcoxon), and the percent of results being positive from 7.7% to 11.0% ($p<0.001$, Pearson chi-squared) from 2018 to 2019, respectively.

Conclusions: The high percentage and cost of VF diagnoses during hospitalization might be reduced by more frequent VF testing/repeat testing in ambulatory units. A conventional training program was followed by modest improvement in the frequency of testing and test positivity. More effective implementation methods, and more precise guidance regarding which patients to test, could improve early diagnosis.

33

CLIMATE-DRIVEN MONTHLY PREDICTIVE MODEL OF COCCIDIOIDOMYCOSIS INCIDENCE IN THE SAN JOAQUIN VALLEY OF CALIFORNIA

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Abstract

Introduction
The San Joaquin Valley of California has some of the highest incidence levels of Coccidioidomycosis in the united states. The number of cases varies by year, which is likely driven in part by changes in seasonal climate conditions. We used climate conditions to create an early warning system for the monthly severity of Coccidioidomycosis incidence in the San Joaquin Valley.

Materials and methods
We used monthly, county-level cases of Coccidioidomycosis from 2000-2015 for model development and 2016-2018 for model testing. We accounted for the delayed response between climate conditions, fungal growth, and case reporting by considering lagged responses between climate variables and disease incidence. We examined climate-disease relationships between both surface air temperature and surface precipitation. We defined the San Joaquin Valley of California as Fresno, Kern, Kings, San Luis Obispo, and Tulare counties. We calculated disease incidence using the total number of cases in these five counties and the total population of this region. We detrended monthly incidence to account for increases in incidence that are less likely related to climate, but more likely related to changes in disease awareness and reporting.

Results
Our predictive model consists of two precipitation variables: the squared term of an 8-month moving average of precipitation that is lagged 5 months prior to incidence, and a 12-month moving average of precipitation that is lagged 13 months prior to incidence. Our model successfully captures much of the monthly variability of disease incidence; compared to observed incidence, our model had an adjusted r^2 of 0.56 for 2000-2015. Our model also captures the seasonal cycle of incidence (r^2 = 0.94). We tested our model by predicting monthly incidence for 2016-2018, to which our model performed with an r^2 of 0.65.

Conclusion
We created a model to predict monthly Coccidioidomycosis incidence in the San Joaquin Valley of California based off of measures of lagged precipitation. This model could be used as an early warning system for public health agencies and physicians to understand the likelihood of a severe Coccidioidomycosis season based on antecedent rainfall, providing up to a five months' notice.

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34

A CROSS-SECTIONAL STUDY OF MORTALITY IN CRITICAL CARE PATIENTS WITH DISSEMINATED COCCIDIOIDOMYCOSIS CONTINUED ON FLUCONAZOLE VS. CHANGED TO AMPHOTERICIN B

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Abstract

INTRODUCTION
Disseminated coccidioidomycosis (cocci) is known to have a high morbidity and mortality. Requiring admission to an intensive care unit (ICU) selects the most severely ill patients of this population. While fluconazole is the most common initial therapy for cocci, patients with severe illness may be changed to amphotericin B if patients worsen on fluconazole. This study compared the outcomes of ICU patients admitted for disseminated cocci that were either continued on fluconazole or changed to amphotericin B.

MATERIALS AND METHODS
After obtaining institutional review board approval, hospitalization data was obtained for patients with an ICD 9/10 diagnosis code for coccidioidomycosis admitted to a critical care unit from 8/1/2004-7/30/2019, at a tertiary care hospital in California's San Joaquin Valley. From patients on fluconazole at the time of ICU admission, a random sample of 15 patients each were selected from those who remained on fluconazole and those who were changed to amphotericin during their ICU course. Day of change in therapy, 30-day mortality and length of stay was compared.

RESULTS
From 8/1/2004 to 7/30/2019, there were 631 hospitalizations of disseminated cocci patients in one of our critical care units. The 30-day mortality of patients who were started on fluconazole and stayed on fluconazole was 18%. The 30-day mortality of the patients who got switched to amphotericin B was 30%. Chi square testing gave a p value of 0.07.

CONCLUSION
Currently there are few studies that have compared outcomes between available treatments for severely ill patients with disseminated coccidioicomycosis. The 2016 IDSA guidelines recommend switching to amphotericin B for azole failure. Our study shows that ICU admission is a marker for high mortality. A mortality rate on fluconazole of 18% indicates a high likelihood of azole failure and that these patients should have been switched to amphotericin. A mortality rate of 30% on those who did switch indicates that if amphotericin is indeed a more effective therapy, these patients should have been changed to amphotericin earlier in the course of their disease.
Further research on the overall mortality vs. survival of all disseminated cocci patients admitted to the ICU is necessary.

35

A RARE CASE OF SYRINGOBULBIA IN A PATIENT WITH COCCIDIOIDOMYCOSIS MENINGITIS

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Abstract

INTRODUCTION
Meningitis is the most extreme and lethal presentation of coccidioidomycosis and is a challenging manifestation to treat. We describe an unusual case of a young Hispanic male whose treatment for coccidioidal meningitis is further complicated by CNS anatomical abnormalities.

METHODS: Retrospective case report.

SUMMARY OF RESULTS:
We report the case of a 20-year-old Hispanic male who was diagnosed with coccidioidal meningitis in Sep 2017. He underwent a ventriculoperitoneal shunt procedure and endoscopic third ventriculostomy shortly after his diagnosis at Children's Hospital Orange County. After failing fluconazole treatment, isavuconazonium sulfate treatment was initiated in 2018. In April 2019, the patient experienced left upper extremity weakness and left chest, arm and face paresthesia. Evaluation by MRIs of the brain and cervical spine revealed Chiari 1 malformation with a cystic cavitary lesion in the upper cervical spine extending into the brain stem with syringomyelia and syringobulbia. The patient underwent a posterior fossa craniotomy with C1 laminectomy and reported improvement of numbness and paresthesia with some residual facial numbness. The patient experienced several months without any unusual symptoms except for morning headaches which improved with over the counter medication. In Sept 2019, the patient developed intermittent double vision, blurry vision, headache and profound fatigue. The patient again underwent MRI which showed a new finding of moderate hydrocephalus without midline shift and suspected shunt failure. The patient underwent a ventriculoperitoneal shunt revision and is currently recovering from the procedure.

CONCLUSION:
The concomitance of coccidioidal meningitis with Chiari 1 malformation, syringobulbia and syringomyelia has not been presented in the literature. Awareness of this possible presentation can aid in faster recognition and treatment and prompt surgical intervention.

36

PULMONARY COCCIDIOIDOMYCOSIS WITH INTRAPARENCHYMAL, OSSEOUS AND CUTANEOUS DISSEMINATION

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Abstract

INTRODUCTION: Coccidioidomycosis (cocci) can disseminate to the central nervous system (CNS), bones, joints, and skin. Intraparenchymal cocci is a rare form of disseminated CNS infection.

METHODS: Retrospective Case Report

SUMMARY OF RESULTS:
A 27-year-old Hispanic male with no past medical history presented with subjective fevers, rigors, night sweats, unintentional weight loss, decreased appetite, nausea, vomiting, photophobia, and constant diffuse headaches of three months duration. Coccidioides (cocci) serology of the cerebrospinal fluid (CSF) showed positive immunodiffusion (ID) IgG and complement fixation (CF) of 1:8. Serum cocci serology demonstrated positive ID IgG, ID IgM, and CF of 1:256. The patient also presented with cutaneous skin lesions along the right lower lip and scalp, excisional biopsy showed Coccidioides spherules with endosporulation. Whole-body bone scan was negative for osseous cocci. The patient was discharged on oral fluconazole 1000 mg daily. Consequently, the patient returned approximately three months later, complaining of the right knee and buttock pain, as well as shortterm memory loss. Neuroimaging revealed Intraparenchymal lesions suggestive of parenchymal cocci. Computed tomography (CT) demonstrated a right middle lobe infiltrate, and a complex multiloculated fluid collection in the right gluteus with sacroiliitis status post drainage of right gluteal abscess with a pigtail catheter left in place. Cultures of the pelvic fluid grew C. immitis. CT findings are consistent with presumptive disseminated cocci to the soft tissue, right posterior iliacus muscle, right flank, spleen, suprahilar, retroperitoneal, and mesenteric lymph nodes. CT also revealed disseminated osseous cocci to L1 vertebra, right hemisacrum, and right iliac bone despite negative bone scan. The treatment plan entailed lifelong fluconazole 1000 mg daily for his meningitis plus liposomal amphotericin B infusions for 12-24 weeks depending on his serological and clinical response.

CONCLUSION: When the host is immunogentically disadvantaged, there is no limit for the degree of dissemination of coccidioidomycosis. Optimal choice and duration of treatment in intraparenchymal dissemination is not clear.

37

A DESCRIPTIVE ANALYSIS OF FLUCONAZOLE UTILIZATION AT 2 ACADEMIC MEDICAL CENTERS IN THE VALLEY FEVER CORRIDOR OF ARIZONA

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Abstract

INTRODUCTION:
Fluconazole is a common antifungal used at hospitals and is an important target for antimicrobial stewardship (AS). Fluconazole is also used for the management of coccidioidomycosis. The objective of our study was to describe fluconazole prescribing patterns at two academic centers in Arizona.

MATERIALS AND METHODS:
We conducted a retrospective analysis of fluconazole usage in adult patients. One month from each quarter in a one-year period (November 2017-November 2018) was selected (4 months in total). All adult patients that received fluconazole at Banner University Medical Center-Tucson (Hospital A) and Banner University Medical Center-Phoenix (Hospital B) in the selected months were identified. Patient demographic information and Charlson comorbidity index (CCI) to quantify the degree of comorbidity were collected. We then analyzed patients in the study by defining the fluconazole usage as directed towards coccidioidomycosis management or non-coccidioidomycosis management (e.g., candidiasis). In the coccidioidomycosis management group, we characterized the initial fluconazole dose during the patient's course as directed for empiric, targeted, or prophylaxis treatment. Finally, we performed a subgroup analysis of the empiric coccidioidomycosis group. The study received IRB approval from our institution.

RESULTS:
During our study period, 1239 pts were included in the analysis. Patient information is shown in Table 1. Overall, most of the fluconazole usage was directed towards coccidioidomycosis management (63.5%, 787/1239). A significant amount of that usage was directed towards coccidioidomycosis prophylaxis at both Hospital A and Hospital B (67.4% (234/347) and 75% (330/440), respectively). In addition, fluconazole usage directed towards empiric coccidioidomycosis management was higher at Hospital A versus Hospital B (18.4% (64/347) versus 9.5% (42/440), respectively). Further patient data for the empiric coccidioidomycosis group is shown in Table 2.

CONCLUSION:
We report the results of a descriptive study that demonstrate that 63.5% of fluconazole usage in adults at two academic medical centers was directed for coccidioidomycosis management. In addition to traditional fluconazole targets for AS, our study highlights coccidioidomycosis prophylaxis in solid organ transplant recipients and empiric coccidioidomycosis management as AS targets in endemic regions. These targets are especially important due to the risk for selection of azole-resistant *Candida* species and invasive molds with increased antifungal exposure.

Table 1: Patient information from Hospital A and Hospital B

	Hospital A (%)	Hospital B (%)
Patients	573	666
Age (Mean)	54	52
Male	273 (47.6)	354 (53.2)
Diabetes mellitus	176 (30.7)	266 (39.9)
Coronary artery disease	70 (12.2)	80 (12)
Congestive heart failure	77 (13.4)	41 (6.2)
Chronic kidney disease	148 (25.8)	98 (14.7)
ESRD	57 (9.9)	132 (19.8)
COPD	47 (8.2)	47 (7.1)
Asthma	19 (3.3)	25 (3.8)
SOT	215 (37.5)	322 (48.3)
Malignancy	150 (26.1)	105 (15.8)
Rheumatology diagnosis	47 (8.2)	70 (10.5)
Cirrhosis	24 (4.1)	76 (11.4)
HIV	16 (2.8)	29 ((4.4)
CCI > 3	356 (62.1)	436 (65.5)
Coccidioidomycosis -directed management • Empiric • Targeted • Prophylaxis	347 (60.6) 64 49 234	440 (66.1) 42 68 330

Abbreviations: ESRD= End-stage renal disease; COPD= Chronic obstructive lung disease; SOT= Solid organ transplant recipient; HIV= Human immunodeficiency virus; CCI= Charlson comorbidity index

Table 2: Patient information for Fluconazole Utilization Directed Towards Empiric Coccidioidomycosis Management at Hospital A and Hospital B

	Hospital A (%)	Hospital B (%)
Patients	64	42
Diabetes mellitus	14 (21.9)	16 (38.1)
COPD	12 (18.8)	5 (11.9)
SOT	4 (6.3)	6 (14.3)
Malignancy	14 (21.9)	6 (14.3)
ESRD	5 (7.8)	5 (11.9)
Chronic kidney disease	3 (4.7)	6 (14.3)
Cirrhosis	2 (3.1)	3 (7.1)
Rheumatology diagnosis	8 (12.5)	4 (9.5)
CCI > 3	37 (57.8)	25 (59.5)

Abbreviations: ESRD= End-stage renal disease; COPD= Chronic obstructive lung disease; SOT= Solid organ transplant recipient; CCI= Charlson comorbidity index

38

EFFICACY AND ASSOCIATED DRUG EXPOSURES OF ISAVUCONAZOLE (ISAV) AND FLUCONAZOLE (FLU) IN AN EXPERIMENTAL MODEL OF COCCIDIOIDOMYCOSIS

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Abstract

INTRODUCTION: *Coccidioides* spp. are important pathogens in endemic regions of the Americas and new treatment options are needed. Isavuconazonium sulfate (ISAVUSULF) was evaluated in a well-established disseminated coccidioidomycosis murine model, and a novel PK-PD coccidioidomycosis murine model designed to characterize drug exposures associated with efficacy for ISAVUSULF and FLU.

MATERIALS AND METHODS: Macrobroth dilution was performed on seven *Coccidioides* spp. isolates over a 2-fold dilution range (0.19 to 100mg/L) to measure minimum effective concentrations (MEC) and minimum fungicidal concentrations (MFC). Mice were inoculated with Silveira strain of *Coccidioides posadasii*. Treatment started 4 days post-inoculation. Model 1 tested therapy of 19 days and 30-day off-therapy observation. Survival and residual fungal burden in lungs, livers, and spleens were measured. Treatments ISAVUSULF [186, 279, or 372mg/kg twice-daily (equivalent to ISAV 100, 150, or 200mg/kg twice-daily)], FLU (20 or 100mg/kg once-daily), and no treatment (UC). Model 2 included a 7-day treatment period with ISAVUSULF [74.4, 111.6, or 148.8mg/kg twice-daily (equivalent to ISAV 40, 60, or 80mg/kg twice-daily)], FLU (20 or 100mg/kg once-daily), and UC. Fungal burden was measured on days 4, 6, 8 and 11 post-inoculation and serial plasma samples obtained 4 and 10 days post-inoculation.

RESULTS: In vitro testing demonstrated fungistatic effects of both azoles (MFC>MEC). In model 1, ISAVUSULF 186mg/kg twice-daily and both FLU doses showed significantly higher survival compared to UC. ISAVUSULF 186 and 279mg/kg twice-daily and FLU 20 and 100mg/kg significantly reduced fungal burden in all organs compared to UC. Toxicity of ISAVUSULF 279 and 372mg/kg twice-daily limited survival comparisons to FLU and UC, as well as CFU comparisons for the 372mg/kg dose. In model 2, >1 $\log_{10}$ CFU/g reduction in fungal burden over time was demonstrated with higher AUCs (56.8 to 75.8mg•h/L) than achieved with 40mg/kg twice-daily (37.9mg•h/L) in the spleen and liver but not the lungs (mean change in $\log_{10}$ CFU/g -0.593 (SD + 0.434)). FLU AUCs, 100 and 500mg•h/L, for 20 and 100mg/kg doses, respectively, >1 $\log_{10}$ CFU/g mean reduction in fungal burden over time was achieved in all organs.

CONCLUSION: ISAVUSULF improved survival and showed significant reductions in fungal burden after prolonged therapy, similar to FLU. In the novel PK-PD model, increasing plasma drug exposures resulted in decreases in fungal burden over time.

39

COCCIDIOIDES DISTRIBUTION CHANGES PREDICTED BY GLOBAL CLIMATE CHANGE MODELS

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Abstract

Exploration of the projected effects of climate changes on the fungal disease coccidioidomycosis (cocci) over the present-2050 time frame is of interest because the causative agent (the soil fungus *Coccidioides spp*) is endemic to dry/desert areas of the Western Hemisphere which are projected to become warmer and more arid in coming decades, with potentially large impacts on dust, the primary dispersal vector of cocci.
I have built a Species Distribution Model (SDM) which combined: (1) geographic range, (2) habitats where cocci is found, and (3) environmental data. *Geographic Range* : Geographic data (state/province and county occurrence) were categorized into four categories: High, Medium, Edge Area, Area not known to have cocci. *Habitat for cocci* : Cocci responds to microhabitat conditions such as pH and salt content of soil as well as larger ecosystem scale variables. The World Wildlife Fund's Terrestrial Ecoregions of the World (2004) was used as a proxy for cocci habitat. *Environmental Data* An ensemble approach was used to investigate the robustness of the SDM model (based on the 17 BioClim variables). Climate data was obtained from 19 different GCM models which support the Intergovernmental Panel on Climate Change (IPCC). 19 different SDMs were created to investigate how sensitive the SDM model was to climate inputs. In addition, four Representative Concentration Pathways (RCPs) were used as predictions of future conditions to investigate how the possible suite of greenhouse gas concentrations trajectories might influence the SDM model output.
Geographic range and habitat data were combined to create pseudo occurrence/non-occurrence points across cocci's range. These points and environmental data were used as inputs to MAXimum ENTropy (MaxEnt) modelling program. The current conditions model predictions were subtracted from the future conditions model to examine how the range of *Coccidioides spp*. is expected to expand or contract in the future and its sensitivity to climate change model parameters. Interestingly, some areas of current endemism are projected to decrease in habitat suitability while large areas of the west (in particular Washington) were predicted to have increased habitat suitability and thus represent potential areas of range expansion.

40

DEFINING CRITICAL GENES DURING SPHERULE REMODELING AND ENDOSPORE DEVELOPMENT IN THE FUNGAL PATHOGEN, *COCCIDIOIDES POSADASII*

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Abstract

INTRODUCTION

The *Coccidioides* genus is the only known fungal pathogen to make specialized parasitic spherules, which contain endospores that are released into the host upon rupture. The molecular determinants involved in this key step of infection remain largely elusive as 49% of annotated genes are hypothetical with unknown function. An attenuated strain *Δcts2/Δard1/Δcts3* was previously created for vaccine development. This strain develops into spherules that do not endosporulate, which prevents completion of the parasitic lifecycle. In this study, we sought to identify pathways active in the wild-type strain during spherule remodeling and endospore formation that have been affected by gene deletion in the mutant. Thus, we compared the transcriptome and volatile metabolome of *Δcts2/Δard1/Δcts3,* to its wild-type parent strain C735.

MATERIALS AND METHODS

Raw reads were aligned to the reference genome using TOPHAT2 and analyzed using the Cufflinks package. Genes of interest were screened in an *in vivo* model using NanoString technology. Next, solid-phase microextraction (SPME) and comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS) was used to collect and analyze the volatile organic compounds (VOCs). Transmission electron microscopy was used to view internal structures.

RESULTS

Our RNA-Seq analyses reveal approximately 280 significantly differentially regulated transcripts that are either absent or improperly up/down regulated in the mutant compared to the parent strain. These genes are related to mitochondrial respiration, nitrogen recycling, and iron assimilation. Within these genes, 13 are hypothetical and specific to the *Coccidioides* genus. HeathFurther, we identified 40 VOCs uniquely produced by the wild-type *C. posadasii* C735. We visualized multiple stages of internal remodeling in the wild-type spherules, whereas the mutant appeared arrested in development.

CONCLUSION

The transcripts which deviated from the wild-type expression pattern would suggest that the genes are tied to networks that are impacted by deletion and may be critical for endospore development and/or spherule rupture in the wild-type strain. Additionally, the unique VOC profile provides evidence that the wild-type strain produces small signaling molecules into the environment during this stage in development. Overall, our results provide the first targeted list of key genes that are active during endospore formation and demonstrate that this approach can define logical targets for functional assays in future studies.

41

AWARENESS AND KNOWLEDGE OF COCCIDIOIDOMYCOSIS IN ARIZONA, 2016

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Abstract

INTRODUCTION

Awareness and knowledge of coccidioidomycosis may be associated with early care seeking and testing. In the 2008 Behavioral Risk Factor Surveillance System (BRFSS), the Arizona Department of Health Services (ADHS) found that 20% of Arizona residents had never heard of valley fever and 36% could not correctly state how it is transmitted. We surveyed Arizona residents about valley fever in 2016 and examined associations between demographic characteristics and symptom recognition.

MATERIALS AND METHODS

Respondents to the 2016 Arizona BRFSS, an annual population-based telephone survey of noninstitutionalized adults about health behaviors, were asked to list symptoms (fever, cough, tiredness) of valley fever and state whether they knew someone who has had valley fever. Weighted estimates and inferential statistics were calculated using survey analysis procedures in SAS v9.4.

RESULTS

5,328 respondents were interviewed, and 4,807 (90.2%) had complete responses available for analysis. Three percent (95% CI: 2.0%, 4.2%) of respondents did not know about valley fever. Of respondents who knew about valley fever, 30.2% (95% CI: 28.0%, 32.5%) knew someone who had valley fever. At least one symptom was identified by 39.3% of respondents. Fatigue was the most commonly identified symptom (21.9%). Only 4.8% (95% CI: 3.6%, 5.9%) correctly identified fever, cough, and fatigue as symptoms of the disease. Respondents who knew someone who had the disease were more likely to identify at least one (OR: 5.7, 95% CI: 4.6, 7.1) and all three symptoms (OR: 4.1, 95% CI: 2.6, 6.6). Recognition of at least one symptom increased with educational attainment and ranged from 15.6% among those without a high school diploma to 55.6% among those with a college degree or higher. Symptom recognition was highest among White respondents (47.3%) and lowest among Black respondents (23.1%).

CONCLUSION

While most Arizona residents knew about valley fever, symptom recognition was limited. Educational campaigns to increase symptom recognition for valley fever should target specific demographic groups.

42

IN VITRO CHARACTERIZATION OF RECOMBINANT VACCINE-INDUCED CELLULAR IMMUNITY AGAINST COCCIDIOIDOMYCOSIS

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Abstract

Introduction: Vaccination against coccidioidomycosis is feasible as patients can develop life-long immunity to infection. We utilized a recombinant antigen (rCpa1) enveloped into gluten-chitin particles (GCP) as an adjuvant-delivery system in order to elicit protective immunity against coccidioidomycosis. Protective immunity to this vaccine in mice is largely dependent on CD4 T cells, especially Th17 cells. Our objective is to characterize human T-cell responses to the newly created vaccine.

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

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

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

43

CHARACTERIZING THE COCCIDIOIDES VOLATILE METABOLOME FOR BREATH TEST DEVELOPMENT

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Abstract

INTRODUCTION: The current diagnostics for Valley fever are severely lacking due to invasiveness and poor sensitivity, contributing to a 23 day median time-to-diagnosis. There is a critical need for sensitive, non-invasive diagnostics for detecting and identifying Valley fever lung infections. Our long-term goal is to substantially shorten the time-to-diagnosis for Valley fever through the development of sensitive and specific breath-based diagnostics. In the near-term, we are working toward identifying volatile organic compound (VOC) biomarkers of *Coccidioides posadasii* and *C. immitis* infections via metabolomics analyses of *in vitro* cultures.

MATERIALS AND METHODS: Six strains of *C. posadasii* (three AZ and three TX/MEX/SA population strains) and six strains of *C. immitis* (three SJV and three SDMX strains) were cultured in triplicate for 96 h in RPMI 1640 with 10% FBS media at 39°C in 10% CO_2 to induce spherule formation, and normoxia at 30°C for mycelial formation. The spent media were filter sterilized for volatile metabolomics analyses by HS-SPME-GCxGC-TOFMS. The metabolomes of each strain under each condition were compared using statistical analyses.

RESULTS: We found that the volatile metabolome of *Coccidioides* is more dependent on growth phase (spherule vs. mycelia) than species. We have identified 39 VOCs that are commonly produced by both species of *Coccidioides* during mycelial growth, 54 during spherule formation, and 9 that are produced by both species under both growth conditions. Controlling for growth phase, we did not observe any segregation between *C. immitis* and *C. posadasii* by their VOCs via principal components analysis. Interestingly, we observed that the widely-studied *C. immitis* RS was a significant metabolic outlier during both growth phases from the 11 other strains of *Coccidioides* we analyzed.

CONCLUSIONS: *The volatile profiles of C. posadasii and C. immitis strains have strong similarities, indicating that a single suite of Valley fever breath biomarkers can be developed to detect both species. However, the uniqueness of the C. immitis RS profile either suggests that it is not a representative strain of the genus, or that there is greater heterogeneity in the Coccidioides metabolome than we captured in our limited sample set.*

44

PROGRESS TOWARD A VALLEY FEVER BREATH TEST: IN VIVO BIOMARKERS FROM A MURINE MODEL

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Abstract

INTRODUCTION: The current diagnostics for Valley fever are severely lacking due to poor sensitivity and invasiveness, leading to delayed diagnosis, inappropriate treatment, lost productivity, and increased medical costs. There is a critical need for sensitive and non-invasive diagnostics for detecting and identifying Valley fever lung infections. Our long-term goal is to develop a breath-based diagnostic for coccidioidomycosis lung infections. Our current objective is to identify and validate volatile biomarkers of *Coccidioides posadasii* and *C. immitis* infections via metabolomics analyses of mouse model lung infections. Herein we present data on the volatile profiles of bronchoalveolar lavage samples from murine lung infections with *C. posadasii* Silveira and *C. immitis* RS, and their relationships to the volatile profiles of each species grown *in vitro* as spherules and as mycelia.

MATERIAL AND METHODS: Three cohorts of female C57BL/6 mice, 6-8 weeks old, were infected by intranasal inoculation with *C. immitis* RS (n=6), *C. posadasii* Silveira (n=6), or vehicle control (n = 4). A dose of 100 conidia in 30 µl sterile PBS was used for infection. The mice were sacrificed at ten days post inoculation. Tracheal intubation followed by PBS washing recovered approximately 1 mL of bronchoalveolar lavage fluid for volatile organic compound (VOC) collection and analysis by headspace solid phase microextraction and comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (HS-SPME-GC×GC-TOFMS).

RESULTS: Preliminary analyses of the VOCs in the murine lavage samples yielded 31 VOCs produced by *Coccidioides* infection *in vivo*, with 17 VOCs produced by both *C. posadasii* and *C. immitis* infections. Only three of these putative *in vivo* biomarkers were also observed *in vitro*, and only in the *in vitro* spherule samples.

CONCLUSIONS: Our pilot data suggest that *in vitro* spherule VOCs are more likely to translate to *in vivo* infections than mycelia cultures, and that there are significant changes in the volatilome when *Coccidioides* is inside a host. Future analyses will focus on identifying infection VOCs from human Valley fever infections, and determining possible biomarkers for discriminating coccidioidomycosis vs. bacterial pneumonia.

45

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

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Abstract

INTRODUCTION:
Previously we described a 3-generation family carrying a single missense mutation in *STAT4* resulting in a GLU to GLY change at AA 626 (E626G). All individuals carrying this mutation disseminated *Coccidioides*. Transfection of DNA encoding 626G into WT human cells resulted in decreased STAT4-mediated cytokine responses. C57Bl/6NJ (B6) mice with knock-in of the homologous mutation have increased susceptibility to *Coccidioides posadasii* (Cp) infection despite normal lymphoid and myeloid compartments, and are protected by vaccination with live, attenuated *Δcps1*.

MATERIALS AND METHODS:
B6 and isogenic E626G mice were inoculated intranasally with 50 spores of a reduced virulence strain, Cp1038. Cellular phenotyping of spleens, lung, and mediastinal lymph nodes and cytokine production in the lungs were determined.

RESULTS:
Fourteen days post infection E626G mice had fewer B cells and activated T cells in the mediastinal lymph nodes. At later times cell composition of E626G and B6 were similar in all organs. Despite similar cell numbers, cells from E626G heterozygote lungs produced less IFN-gamma on days 28, 35 and 42 after infection. IFN-gamma-stimulated cytokines (IP-10, MIG, MIP-2) were also significantly reduced compared to infected B6 mice. In other experiments, we crossedE626G to both B6 and B6-*Stat4*$^{null/null}$ to create heterozygotes for E626G as well as crossing B6 to B6-*Stat4*$^{null/null}$ to create Stat4 hemizygotes to account for gene dosage. All F1 mice containing an E626G allele were equally more susceptible to Cp1038 infection as compared to Stat4$^{null/+}$ and WT B6 who were also the same. Thus, the E626G mutation acts as a dominant negative.

CONCLUSION:
E626G mice have reduced IFN-gamma production and transiently reduced accumulation of immune cells in the draining lymph node following Cp1038 infection. These findings provide immunologic detail to their phenotype and suggest an explanation for why this STAT4 mutation in humans caused DCM is a dominant negative.

46

TUBERCULOSIS PERSPECTIVES ON VALLEY FEVER: THREE EXAMPLES TO HIGHLIGHT CLINICAL POINTS OF INTEREST

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Abstract

INTRODUCTION

Tuberculosis (TB) and coccidioidomycosis (cocci) are often considered differential diagnoses. Through case monitoring, the Arizona Department of Health Services (ADHS) TB program observed cocci to be a reported comorbidity among TB patients. Therefore, ADHS performed a data crossmatch between TB and cocci in Arizona. Three case examples highlight the TB perspective on the overlap between TB and cocci in Arizona.

MATERIALS AND METHODS

Data crossmatch of reported cocci and TB in Arizona from 2009 to 2016. Case studies extracted from TB surveillance data.

RESULTS

There is value for TB programs in "thinking cocci" when "thinking TB". In Arizona, cocci is more common than TB; in 2018, the reported number of cocci cases was forty-four times greater than TB (7,636 vs. 178). Example: Southeastern state contacted ADHS regarding commercial pilot presenting with TB symptoms and cavitation on X-ray. After TB/cocci workup, no public health interventions were needed preventing unnecessary travel restrictions and contact investigations.

Cocci diagnosis does not exclude TB disease. Nine-percent (157/1,745) of TB cases in the data crossmatch were also reported to have cocci. The TB case rate among reported cocci cases was 204.7/100,000, sixty-two times higher than Arizona's 3.3/100,000 case rate. Example: MTB isolated from sample of US-born white male who underwent a LUL lobectomy. His only TB risk factor was retirement from overseas military service, over ten years before symptom onset. Medical history included nine months of declining health despite fluconazole treatment.

TB, even with concurrent cocci, is curable. While dual diagnoses were more likely to be cavitary (OR 1.65; 95% CI:1.16-2.35) and/or miliary (OR 2.58; 95% CI:1.40-4.76) on chest imaging, no difference was found in TB treatment outcomes. Example: patient with disseminated TB and cocci cured of TB.

CONCLUSION

Cocci and TB can be comorbidities as well as differential diagnoses. Diagnosis with one does not exclude the other. Working patients up simultaneously for cocci and TB is of benefit to the patient and public health. For cocci patients who do not respond to treatment, TB workup with sputum collection could be of added value. TB, even with concurrent cocci, is curable.

47

A CASE OF COCCIDIOIDOMYCOSIS DISSEMINATION TO THE SMALL BOWEL

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Abstract

Disseminated coccidioidomycosis is rare. Dissemination to the small bowel is even more exceptional, with less than 10 cases described in the literature. We present a case of coccidioidomycosis involving the small bowel in an immunocompetent young male.

A 36 year-old Japanese male farmer from Visalia, CA presented to Gastroenterology service with abdominal pain, postprandial bloating and unintentional weight loss for 6 months. Besides abdominal tenderness, initial exam revealed a right sided neck mass, measuring 5 x 5 x 1 cm by ultrasound. Esophagogastroduodenoscopy (EGD) revealed a severe duodenal stricture concerning for malignancy warranting inpatient work up. Computed tomography (CAT) of the lungs found multiple lung nodules with extensive mediastinal and hilar adenopathy. Neck CAT scan revealed ulcerated neck mass with gas focus while abdominal scan revealed ascites and soft tissue density infiltrating the porta hepatis, encasing the main portal vein along with peritoneal enhancement. A biopsy of the neck mass and the duodenal stricture were performed. Histopathology of both tissue sites revealed fungal elements with cultures positive for *Coccidioides immitis*. Serologic testing by immunodiffusion (ID) was positive for IgG. Quantitative ID resulted in a titer of 1:128. CSF and ascitic fluid testing were unrevealing. The patient was treated with fluconazole 600 mg oral daily. After 4 months of therapy, his symptoms resolved; repeat EGD showed resolution of duodenal stricture, and repeat imaging showed only improving residual lung nodules.

Risk factors such as race, ethnicity, diabetes and immunocompromised states are known to contribute to the progression of coccidioidomycosis but specific triggers for its dissemination are still unclear. Our patient is unique because he did not have these risk factors; yet, he developed a widely disseminated disease including the small bowel. This case reiterates the importance of early consideration for coccidioidomycosis in endemic area even in patients without the usual risk factors. Early identification and recognition of the disease are important to attain early therapy and disease control. Further research is warranted to understand mechanisms that predispose patients to getting certain organ dissemination.

48

A CASE OF SPONTANEOUS CEREBELLAR ECTOPIA IN A PATIENT WITH COCCIDIOIDAL MENINGITIS

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Abstract

INTRODUCTION
Spontaneous cerebellar ectopia without acute trauma is rare. Meningitis has not yet been reported as a cause in the literature. We present a case of coccidioidal meningitis complicated by cerebellar ectopia.

METHODS
Retrospective case report

RESULTS
A 22 year old Hispanic female with five year history of coccidioidal meningitis complicated by non-communicating hydrocephalus requiring ventricular peritoneal shunt with multiple revisions who is on posaconazole and biweekly intrathecal amphotericin B deoxycholate through an Ommaya reservoir presented with acute encephalopathy. Two days prior to presentation, she had lumbar puncture for prognostic studies. She developed new onset headache, confusion and then somnolence, for which family brought patient to hospital. Imaging suggested shunt malfunction due to worsening hydrocephalus as the distal VP shunt was looped around the bladder. She developed fever upon admission. VP shunt and Ommaya were removed and an external ventricular device was placed. Samples from VP shunt grew *Enterococcus faecalis*. The following day she become obtunded requiring intubation. Neuroimaging found obstructive hydrocephalus with compression on each side of the brainstem and downward pressure on the cerebellar tonsils, which extend 13mm below the foramen magnum. She exhibited right sided decorticate and left sided decerebrate posturing as well as right medial gaze palsy. EEG was consistent with coma. EVD drainage was decreased from 20mmHg to 5mmHg and patient was placed in head-down position. She did not show any signs of improvement and 8 days later new VP shunt was placed. The following day she began to track with eyes and follow simple commands. One week later tracheostomy was placed and due to VP shunt malfunction underwent another revision. PEG was placed. After 23 ICU days, patient recovered enough to be stable for telemetry floor. After 6 days, she was transferred to acute rehab. At four months follow up, patient has no neurological deficits, performing all ADLs and is able to drive a vehicle.

CONCLUSIONS
Cerebellar ectopia is not common and when it occurs the sequalae is permanent and prognosis is very poor. Spontaneous appearance and resolution without neurological sequalae is rarely seen and the mechanism is unknown.

49

COCCIDIOIDAL MENINGITIS AND CIRRHOSIS: IS THE GAME OVER?

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Abstract

INTRODUCTION
Coccidioidal meningitis requires lifelong, potentially hepatotoxic, triazole therapy. Complicated cases might need neurosurgical shunt or reservoir for intrathecal treatment. Cirrhosis complicates treatment options due to impaired hepatic function, coagulopathy and thrombocytopenia. We share a challenging case of coccidioidal meningitis in a cirrhotic patient with thrombocytopenia.

METHODS
Retrospective case report

RESULTS
62 year old Hispanic male with alcoholic cirrhosis, portal hypertension requiring transjugular intrahepatic portosystemic shunt (TIPS), thrombocytopenia with splenic embolization, protein S deficiency with thrombosis of portal vein and left cephalic vein now off warfarin for two years, prediabetes and pulmonary coccidioidomycosis for two years off fluconazole for two months due to insurance presented with six weeks of progressive persistent occipital headaches, photophobia, nausea, vertigo, tinnitus, decrease hearing, blurry vision and short term memory lapses. Initial CT neuroimaging found 8mm hyperdense focus in the posterior left cerebellum. Platelets were 51,000/uL. Presenting cirrhosis prognostic scores were Child Pugh A and MELD 10. TIPS was non-functional by ultrasound. MRI brain found mild hydrocephalus and abnormal periventricular signal in midbrain structures with severe leptomeningeal enhancements. Lumbar puncture had normal opening and closing pressures, lymphocytic pleocytosis, hypoglycorrhachia, and elevated protein. Serum and CSF coccidioidal antibody immunodiffusion were reactive with severely elevated complement immunofixation titers. Due to thrombocytopenia intrathecal amphotericin was deferred. Because of fluconazole failure and voriconazole hepatotoxicity, isavuconazole was started. Dexamethasone taper was added due to hearing and short term memory loss. Outpatient audiogram and liver function monitoring was arranged with plan of lumbar puncture in four weeks. The pharmacokinetic and pharmacodynamic impact on his lifelong azole therapy from TIPS recanalization and impaired cytochrome P450 3A4 and 3A5 is unknown.

CONCLUSIONS
Coccidioidal meningitis is fatal if not treated. Hepatic impairment complicates management due to therapeutic limitations. Prognostic impact needs to be studied further but, in our experience, it is diminished.

51

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

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Abstract

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

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

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

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

52

COCCIDIOIDOMYCOSIS AND THE FIVE RASHES

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Abstract

Background
Gifford and Dickson in 1936 acknowledged the skin manifestations of Coccidioidomycosis (CM) as a related but "benign" presentation of coccidioidal infection. Our understanding has been built on this foundation.
Five known types of presumably immune-mediated rashes have been documented with acute pulmonary coccidioidomycosis. This does not represent disseminated disease but rather a host response to what is usually a pulmonary infection. Some of these are relatively common. Most are rare.
The reactive or immune-mediated rash types are Erythema Nodosum, Erythema Multiform, Morbiliform, Uticaria and Erythema Sweetobullosum.
Skin manifestations may be a clue to the diagnosis of a pneumonic presentation. Awareness of the cutaneous manifestations of primary CM can impact clinical diagnosis, therefore familiarity with these entities is significant.
We present a library of Coccidioidal rash images, describe their associated course and possible treatment.

Methods
This study was approved by the Kern Medical Institutional Review Board. Photographic approval was obtained by all patients.

Results
Selected cases with common and less common rashes associated with CM are presented here. Erythema Nodosum is relatively common. Less common are Erythema Multiform, Morbiliform, Uticaria and Erythema Sweetobullosum. Representative photographs are presented.

Conclusion
Primary CM most commonly does not present with cutaneous manifestations. Cutaneous manifestations that have been described are pictured above. Antifungal therapy has no significant effect on the course of any of these cutaneous presentations of CM. Short course, low dose glucocorticoids may be helpful in severe Erythema Nodosum. Specific treatment is probably not available for the other cutaneous reactions to CM disease.

53

COCCIDIOIDAL PERITONITIS: A REVIEW OF 11 CASES

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Abstract

Background

Coccidioidomycosis (CM) is an endemic fungal infection that is found in the southwestern United States and adjacent areas of Mexico as well as Central and South America. In the United States, 150-300 thousand infections occur annually. The majority (60%) are asymptomatic. Of the symptomatic cases, the majority are primary pneumonic disease that varies from mild to very severe. A minority of persons develop disseminated (extrapulmonary disease). The majority of these are meningitis, osteomyelitis, joints and integumentary. However, CM has been described in virtually every part of the body.

We herein describe 11 cases of peritoneal coccidioidomycosis, the demographics, comorbidities, clinical presentation, laboratory, imaging, and pathology results. Treatment is also described.

Methods

This study was approved by the Kern Medical Institutional Review Board. Eight cases were derived from Kern Medical, one from Cedars-Sinai and two from Ventura County. ICD 9 and ICD 10 codes were applied to Kern Medical's electronic health record for retrospective review of ten years. Cases were also submitted by our colleagues in Gynecology and General Surgery. We excluded cases with inadequate data for diagnosis or analysis. We also excluded Coccidioidal peritonitis that was secondary to Ventriculoperitoneal shunt for CM.
Diagnosis is based on a compatible clinical illness, with or without imaging plus histopathologic and/or serologic confirmation.

Results

Eleven records that met the above criteria were recovered. The average patient age was 39 years. Sixty four percent were male. Forty percent were Hispanic, 36% were African American and 18% were Caucasian.
Five of 11 cases (40%) had a prior history of CM. Nine of 11 (82%) presented with nonspecific abdominal complaints. Seven of 11 cases (64%) presented with ascites, five of 11 (45%) had abdominal distension, three of 11 cases (27%) had weight loss and three of 11 (27%), had nausea/vomiting. Three of 11 cases (27%) had an abdominal Cat scan with radiographic findings of "thickening", "studding" or "caking".
Paracentesis was performed in five of 11 cases (45%) patients, two of five cases (40%) were culture positive for C. immitis. All 11 cases were serologically positive by immunodiffusion and complement fixation.

Conclusion

Peritonitis is an uncommon manifestation of coccidioidomycosis. It may be an asymptomatic manifestation found incidentally at surgery or on surgical pathology. The most common presentation is vague abdominal pain with or without ascites. Imaging may demonstrate "omental caking" as typical of carcinoma.

Treatment is usually with an azole, typically fluconazole, high dose. Only rarely is amphotericin B considered. A three-year minimum treatment course is recommended, assuming clinical and serologic response.

54

ISAVUCONAZOLE IN RESCUE THERAPY FOR MENINGEAL AND NONMENINGEAL COCCIDIOIDOMYCOSIS

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Abstract

INTRODUCTION

Coccidioidomycosis (CM) is a dimorphic fungus prevalent in the Southwestern United States. It has protean clinical manifestations in an incidental host, homo sapiens. The therapeutic armamentarium previously available includes polyenes and other azoles. There is in vitro and in vivo data suggesting Isavuconazole (ISAV) is effective in CM. ISAV is marketed as the prodrug Isavuconazonium. This study evaluated the use of ISAV as rescue therapy for patients that have previously been treated with Fluconazole and/or other azole antecedents.

MATERIALS AND METHODS

Approval of Institutional Review Board Kern Medical (KM) was obtained. A retrospective chart review of fifty two patients with CM treated in part with ISAV was undertaken. Forty one met the criteria for evaluation. Twenty five had nonmeningeal (NM) CM and sixteen had meningeal (M) CM.

NM CM included pulmonary and disseminated cases refractory to primary treatment, largely fluconazole. The meningitis patients similarly represent primary azole failures. The patient records were abstracted for NM and M with scoring by the MSG 2020 score system. Scores were taken at disease onset when first seen at KM, at change in therapy to ISAV and at approximately eight months post ISAV. Responders were defined as a reduction of ≥ 50% in NM CM and ≥ 40% for M CM. Partial responders were defined as a reduction of 25-49% for NM CM and 20-39% for M CM.

RESULTS

Of the 25 cases of NM CM, (8/25) 32% were responders and (6/25) 24% were partial responders. Of the 16 cases of M CM, (8/16) 50% were responders and (2/16) 13% were partial responders.

CONCLUSION

This study suffers from retrospective non-randomized design. Therapeutic challenges still exist for refractory CM. Since this is an uncontrolled retrospective study, it cannot be assured that patients wouldn't have improved by continuing their previous azole therapy. Eight months ISAV for patients that have already failed on one azole may not be adequate duration for proper assessment.

This study suggests that ISAV may be an additional azole that is effective in refractory CM. ISAV may also prove to be an effective rescue therapy. ISAV can be useful in treating a broad range of patients with CM. It would appear to be an improvement over prior standard therapies for both non-meningeal and, in particular, meningeal CM.

55

CAVITARY COCCIDIOIDOMYCOSIS IN DIABETICS

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Abstract

Background:
Pulmonary Coccidioidomycosis (PCM) is endemic to the Southwestern United States and Mexico. Its clinical manifestations vary depending on extent of infection and immune status of the host. Many infections are common and complicated in persons with diabetes. This may be caused by any number of immune alterations.[1] People with diabetes mellitus (DM) are more likely to experience severe coccidioidomycosis[2] and cavitary lung disease is common.[3]
Cavitation in these patients represents chronic disease and clinicians use size, location, wall thickness, and number to characterize them. The purpose of this study is to evaluate pulmonary cavitary coccidioidomycosis in DM.

Methods:
Approval was obtained from the Institutional Review Board, Kern Medical. A retrospective chart review was conducted on the records of patients with proven PCM and DM. We evaluated the records for radiographic reports, chest x-rays (CXR), and computed tomography (CT) imaging for these patients and assessed their cavitary lesions. The location, number, and size were recorded.

Results:
We reviewed the imaging for 110 diabetic patients with PCM and found 52/110 (47%) patients with at least 66 cavities, as 14/52 (27%) patients had multiple cavitations. 38/66 (58%) lesions were found in the upper lobes, 25/66 (38%) in the lower lobes, and 3/66 (4%) were right middle lobe lesions. 33/66 (50%) cavities were located the right lung, and 33/66 (50%) localized in the left lung.

We defined size of the lesion as the single greatest dimension, when known. The size of cavities varied greatly, ranging from 7mm to 60mm, with a mean size of 26.5mm. The median value was 25mm, and the mode was 21mm.

The American Diabetic Association classifies controlled Diabetes with a glycosylated hemoglobin (HBA1c) of < 7.0%. 46/52 (88%), of our diabetic population had uncontrolled diabetes, 2/52 (4%) had controlled diabetes. We were unable to determine glucose control for 4/52 (8%) of our patients.

Conclusion:
Cavitary disease is substantially more common in uncontrolled diabetic patients than in the typical population of individuals with primary pulmonary coccidioidomycosis. Efforts to improve glycemic control in diabetic patients may be of value in preventing progression to cavitary pulmonary coccidioidomycosis.

56

ENZYMATIC AND SEROLOGIC DIFFERENCES TO FUNGAL AND MAMMALIAN-PRODUCED RECOMBINANT *COCCIDIOIDES* ANTIGENS.

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Abstract

INTRODUCTION: Serological detection of Valley Fever (VF) employs mycelial-phase culture filtrates as antigen. While culture filtrates are thought to provide the most specific diagnostic antigen, preparation includes the growth of large volume *Coccidioides* cultures which require employment of extensive safety precautions in a BSL3 setting. An additional concern with use of culture filtrates as an antigen source is batch variability, as expression of immunogenic proteins within each lot are variable. To address safety and batch variability concerns, we propose the use of recombinant *Coccidioides* proteins as a consistent and reliable antigen source.

MATERIALS AND METHODS: Initially, we expressed two proteins in HEK-293F cells known to be serologically reactive and secreted in mycelial phase *Coccidioides* cultures, CTS1 and HL-Ag. Next we expressed CTS1 and HL in *Uncinocarpus reesii* (*U.reesii*), with the hypothesis that fungal-produced antigens would be more serologically reactive than those expressed in a mammalian system. Using *Agrobacterium*-mediated transformation methods, we established a binary vector system termed fevUr, in which His_6-tagged recombinant antigen could be readily produced. To date, we have cloned and expressed CTS1 and HL in *U. reesii* and tested serological reactivity of 100 VF patients and 100 non-infected donors to CTS1 expressed in mammalian and fungal expression systems.

RESULTS: We characterized differences in enzymatic and serologic reactivity of fungal and mammalian-expressed CTS1 antigen. Enzymatically, *U.reesii*-produced CTS1 (Ur-CTS1) was active on both 4-Nitrophenyl-N,N'-diacetyl-ß-chitobioside (4NDBC) and 4-Nitrophenyl-ß-N-acetyl-D-glucosaminide (4NBAG), whereas 293F-CTS1 was active only on 4NDBC substrate. Surprisingly, 293F-produced CTS1 (293F-CTS1) was more serologically reactive than Ur-CTS1 in 86 of 100 of VF sera tested by ELISA, but 4 of 100 patient sera reacted more strongly to Ur-CTS1. The remaining 10 VF sera tested demonstrated approximately equal reactivity to both recombinant antigens. Sensitivity of 293F-CTS1 and Ur-CTS1 was determined at 90% and 86%, respectively. Specificity of both 293F-CTS1 and Ur-CTS1 was determined at 82%.

CONCLUSION: Contrary to our original hypothesis, 293F-produced CTS1 was more serologically reactive in ELISA. At this point in time, it is unclear why the majority of patients reacted more strongly with mammalian CTS1 than with fungal CTS1, especially from *U. reesii*, the closest fungal relative to *Coccidioides*. Despite puzzling differences in reactivity, comparable specificity and slightly increased sensitivity of 293F-CTS1 suggest promising diagnostic utility of recombinant CTS1 as a consistent and reliable antigen source.

57

AN ASYMPTOMATIC PRESENTATION OF DISSEMINATED COCCIDIOIDOMYCOSIS TO THE ADRENAL GLANDS

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Abstract

Background
Disseminated coccidioidomycosis to the adrenal glands (AG) has been rarely described. We present a patient with melanoma found to have bilateral thickening of the AG on computed tomography (CT) imaging done for surveillance of metastasis. Biopsy of the AG demonstrated spherules consistent with coccidioidomycosis, and the patient was treated with fluconazole

Case presentation
A 59-year-old Caucasian-white male with past medical history of sarcoidosis, heart failure, atrial fibrillation, mitral-valve repair, and diverticulosis was diagnosed with melanoma in October 2018. In May 2019, he underwent CT imaging to screen for metastatic lesions that revealed increased nodular thickening of bilateral AG.
A tissue biopsy revealed Coccidioides spherules on Grocott's Methenamine Silver (GMS) stain. Serum Coccidioides Immunodiffusion test (IMDF) was reactive and serum Coccidioides complement fixation (CF) titer was 1:32 (reference range, detectable > 1:2).
At the time of diagnosis, he denied having any symptoms of cough, shortness of breath, new skin lesions, joint pain, or headaches. He was not on any immunosuppressive medications. White blood cells count was 10,400/µL with no remarkable eosinophilia. Serum adrenocorticotropic hormone was 23 pg/ml (reference range 7 to 69 pg/ml) and AM cortisol of 13.9 ug/dL (reference range 4.5 to 22.7). Patient was started on treatment with fluconazole.
Patient presented with visual complaints secondary to optic disc edema three months later. Magnetic resonance imaging of the brain and a lumbar puncture did not suggest any findings for dissemination of coccidioidomycosis to the eye or brain.
A repeat CT obtained six months on treatment suggested moderate improvement of AG thickening. Except for a presentation with visual complaints, the patient did not report any new or worsening symptoms during the course of his treatment.

Discussion
The prevalence of primary adrenal insufficiency is estimated to be 117 per million. Our review of literature revealed only a few case descriptions of disseminated coccidioidomycosis of the AG. In autopsy studies done on disseminated coccidioidomycosis, AG involvement is reported to occur in 17-36% of the cases. The true prevalence may be higher and dissemination to the adrenal glands is likely under-diagnosed.

58

IN SEARCH OF NEW THERAPEUTIC TARGETS DOWNSTREAM OF *CPS1*: IDENTIFICATION OF CPS1 PROTEIN-PROTEIN INTERACTIONS

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Abstract

INTRODUCTION

The current live attenuated vaccine being developed for dogs was created by a full deletion of the *CPS1* gene. This gene, an ortholog of a virulence factor in the pea pathogen *Cochliobolus heterostrophus*, encodes a member of the Dip2 group of the adenylate-forming domain class I super family of proteins present in animals and fungi. Cps1, and related proteins, contain three conserved domains, an N-terminal DMAP1 binding domain, and two adenylate-forming domains. The DMAP1b domain is a protein-protein interaction domain that, in different organisms, binds to the DNA methyltransferase associated protein (DMAP1), a transcriptional co-repressor.

To gain insight on the function of Cps1 in *Coccidioides*, we are screening for proteins that interact with the DMAP1b domain by yeast-2-hybrid (Y2H) techniques.

MATERIALS AND METHODS

The bait construct encodes the 63 amino acids of the DMAP1b domain cloned in frame into the pGBKT7-BD bait vector.

Two mixed condition cDNA libraries were constructed from *C. posadasii* strain Silveira polyA+ RNA. The RNAs were isolated from spherules grown *in vitro* in RPMI at 37 °C with 20% CO_2 for 24, 48, 72, and 96 h, and mycelial cultures were grown for 24 and 48 h in 2XGYE at 37 °C. The two libraries differed in whether the first-strand cDNAs were synthesized using oligo$(dT)_{15\text{-}18}$ or oligonucleotide hexamers as primers. The cDNAs were cloned into the prey vector pGADT7-AD.

Both cDNA libraries were combined and mated with the DMAP1b yeast bait strain to screen for activation interactions.

RESULTS

Forty-four cDNA clones were isolated that complemented the Y2H three-marker selection and the screen for activation of alpha-galactosidase and encode putative interacting proteins. Twenty-two clones were validated as in-frame and unique. These clones correspond to genes encoding: a dimethyl adenosine transferase, an AGC/PKC protein kinase, an SVP1-like protein, the RNA-binding protein NOB1, several ribosomal proteins, as well as a number of hypothetical proteins.

CONCLUSIONS

Definition of Cps1-interating proteins is the first step in defining the role of Cps1 in the biology of *Coccidioides*. Further analysis to verify interactions and thus identify targets for downstream analysis is underway.

59

IDENTIFICATION OF GENOMIC REGULATORY ELEMENTS ASSOCIATED WITH COCCIDIOIDES IMMITIS DIMORPHIC STATES

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Abstract

Introduction: Coccidioides are important dimorphic fungal human pathogens. Little is known about regulation of gene expression and especially how morphology-specific gene expression is controlled.
Methods: To gain insights into how the genomic regulation of transcription in each Coccidioides phase is controlled, we profiled the transcriptome and regulatory landscape of C. immitis RS mycelia and spherule states by strand-specific total RNA-seq, capped-small RNA-seq (csRNA-seq), and small RNA-seq (sRNA-seq).

Results: RNA-seq, together with csRNA-seq, which maps active sites of transcription initiation (TSS) independent of RNA stability at base pair resolution, improved the annotation of full-length transcripts, TSS, and regulatory elements in both mycelia and spherules. RNA-seq identified >7000 transcripts expressed by spherules or mycelia, of which >30% were differentially regulated between phases. Comparatively, csRNA-seq identified more than 20k actively transcribed regions. Most active TSS sites are bidirectionally transcribed (>90%) and roughly 22% percent of TSS clusters initiate 'unstable' transcripts. While only 11k TSS are expressed in mycelia, spherules have an increase of >7k TSS clusters (> 60% increase). The majority of these spherule-specific TSS were promoter-distal initiation sites suggestive of enhancer transcripts (eRNAs) that may regulate gene expression. Spherule-specific TSS were selectively enriched with a WOPR DNA motif which suggests that a Wor1-like transcription factor is involved in spherule-specific gene regulation. In addition to identifying known tRNAs, snRNAs and other non-coding RNAs (ncRNAs), sRNA-seq identified many unknown putative ncRNAs and what appears to be RNA degradation products from overlapping antisense transcripts suggestive of RNA interference.

Conclusions: These data sets improve Coccidioides annotation, identify potential transcriptional regulators of phase-specific gene expression and provide evidence for multiple previously unappreciated mechanisms by which Coccidioides may regulate gene expression for which future mechanistic exploration is warranted.

60

ANALYSIS OF COCCIDIOIDOMYCOSIS AND TUBERCULOSIS IN ARIZONA, 2013–2018

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Abstract

INTRODUCTION
Coccidioidomycosis (cocci) and tuberculosis (TB) are reportable diseases in Arizona and case reports are stored in the Arizona Department of Health Services' (ADHS) Medical Electronic Disease Surveillance Intelligence System (MEDSIS). These diseases can have similar if not indistinguishable clinical and radiologic presentations. Because of these similarities and the high incidence of cocci in Arizona, we investigated the frequency of TB among reported cocci cases, cocci cases among reported TB cases, TB-cocci coinfections, and the timing of report date for each disease among reported coinfections.

MATERIALS AND METHODS
We matched all incident cocci and TB cases reported to ADHS during 2013–2018. TB cases were restricted to those who had active TB infection and were counted in Arizona. This produced three groups: matched (TB-cocci) cases, cocci-only, and TB-only cases. Within the TB-cocci group are three subgroups: cases where cocci was reported first to public health, cases where TB was reported first, and cases with both diseases reported on the same day. We compared case rates and analyzed trends in timing of date reported to public health across the three groups and within the matched cases subgroups.

RESULTS
There were 39,571 cocci cases and 1,129 TB cases reported to ADHS during the study period and the matching process resulted in 39,507 cocci-only cases, 64 TB-cocci cases, and 1,065 TB-only cases. The rate of reported cocci among patients reported to have TB is 5,669 per 100,000 population compared to the rate of reported cocci case of 96.6 per 100,000 in the general population in Arizona during the same time frame. The rate of TB among patients reported to have cocci is 161 per 100,000 population compared to the TB rate of 2.76 per 100,000 in the general population. Of the 64 TB-cocci cases, 22 (34.4%) were reported within two weeks of one another, 37 (57.8%) were reported within 30 days, and 50 (78.1%) were reported within a year. Two of the TB-cocci cases had both morbidities reported on the same day, 38 (59%) had cocci reported to public health first, and 24 (37.5%) had TB reported first.

CONCLUSION
Concurrent coccidioidomycosis and TB case reports are uncommon in Arizona. Further analysis of the clinical and laboratory characteristics of these cases is needed.

61

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

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Abstract

Introduction. Coccidioidomycosis (Valley Fever) is a disease of humans and animals caused by species of the dimorphic fungus *Coccidioides*and is highly endemic to arid regions of the southwestern United States. Recently, Taylor and Barker reviewed modern studies that support the hypothesis that small mammals provide an environmental reservoir for species of *Coccidioides*. This hypothesis is supported in part by the fact that *Coccidioides*genomes have a reduced number of genes associated with plant cell-wall degradation and an increased number of genes associated with animal pathogenesis. We are taking a multifaceted approach to study the lung mycobiome of southwestern wild rodents to explore the distribution and ecology of species of *Coccidioides*.

Methods. Ultrafrozen lung tissues have been obtained for five families of rodents across the southwestern U.S. from UNM Museum of Southwestern Biology and UC Berkeley Museum of Vertebrate Zoology. A combination of next-generation sequencing approaches and culturing have been used to examine the lungs for fungi.

Results. To date, we have examined the lungs of approximately 40 small mammals usingIllumina ITS2 sequencing. An additional 40 small mammals have been examined for live fungi by plating onto agar media. Sequences for species of *Coccidioides* were present in 50% of the samples, albeit often in low relative abundance. Sequence similarity points to the presence of *C. posadasii*in rodent lungs from New Mexico and Arizona, reflecting results obtained from analyses of clinical isolates. Plated fragments from most lung tissues produced one or more fungal colonies. In one experiment employing lung samples from southern California, more than a third produced colonies of *Blastomyces parvus*(*Emmonsia parva*).

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

62

CLUSTER OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS SECONDARY TO SEVERE AND FATAL COCCIDIOIDOMYCOSIS — MARICOPA COUNTY, ARIZONA, JULY–OCTOBER, 2019

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Abstract

INTRODUCTION: On November 18, 2019, Maricopa County Department of Public Health (MCDPH) was notified by two local hospitals of four patients with coccidioidomycosis infection and suspected hemophagocytic lymphohistiocytosis (HLH). HLH secondary to coccidioidomycosis infection has been described in a case report, however to our knowledge, a cluster has not. MCDPH investigated the cluster to determine common exposures and risk factors.

MATERIALS AND METHODS: Medical records were reviewed to determine patient characteristics (demographics, underlying medical conditions); coccidioidomycosis onset, symptoms, time to diagnosis, and dissemination; treatment; HLH diagnostic criteria; and illness severity (intensive care, mechanical ventilation, or extracorporeal membrane oxygenation [ECMO]). Cases addresses were mapped.

RESULTS: All patients were male; one (25%) was Asian/Non-Hispanic, one (25%) was black/non-Hispanic, one (25%) was white/Hispanic, and one (25%) was white/non-Hispanic. Three (75%) had no past medical history and one (25%) had medication-controlled diabetes mellitus type 2. Median age was 31 (range: 20–46) years. No geographic clustering was evident.

Illness onset was during July 15–October 1. Commonly reported symptoms were weight loss/decreased appetite and fatigue (100%), cough and dyspnea (75%), and rash (50%). All patients were diagnosed with coccidioidomycosis during hospital admission, which occurred a median of one day (range: 0–2 days) after admission and 28 days after symptom onset (range: 6–61 days). Three patients (75%) had disseminated coccidioidomycosis. All patients were admitted to the ICU, required mechanical ventilation and vasopressors; three (75%) required ECMO; three (75%) died. All were treated with antifungals, including: liposomal amphotericin B (100%), fluconazole (75%), voriconazole (50%), micafungin and posaconazole (25%). Three (75%) were prescribed steroids for ≥ 14 days in the month before admission.

HLH was suspected during hospitalization in three patients (75%), all of whom met ≥5 HLH diagnostic criteria, and in one patient (25%) post-mortem who did not meet ≥5 criteria.

CONCLUSION: This cluster of four patients with severe/fatal coccidioidomycosis and suspected HLH were previously healthy, young men with no common exposure or risk factor to explain the development of HLH secondary to coccidioidomycosis. Interviews and genetic testing are planned to explore this cluster further.

63

USING GWAS TO DISSECT COCCIDIOIDES BIOLOGY

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Abstract

INTRODUCTION: We aim to map genes underlying a variety of traits in the fungus *Coccidioides*, which causes San Joaquin Valley Fever (VF) using genome-wide association studies (GWAS). Our approach harnesses natural genetic variation between distinct *Coccidioides* strains as a tool in a genome-scale screen for genes that underlie growth and virulence attributes.

MATERIALS AND METHODS: We acquired a panel of distinct *Coccidioides* strains from the collection of Bridget Barker at Northern Arizona University and subjected these strains to sequencing, phenotypic profiling, and genome analysis.

RESULTS: We have sequenced and analyzed the genomes of approximately 75 *Coccidioides* isolates and have shown that they are likely to represent one interbreeding *Coccidioides* population. We are now assaying each isolate for fungal response to stresses found in the host environment, interaction with host immune cells, and the key developmental conversion from infectious arthroconidia to spherule. We have created a facile pipeline to identify single nucleotide polymorphisms that are associated with phenotypes of interest.

CONCLUSIONS: We have developed tools to perform GWAS on *Coccidioides* isolates. This approach will allow us to use natural variation to identify alleles that are associated with phenotypes of interest. Ultimately, we will validate the function of individual genes that are implicated in this analysis by making gene disruption and/or allele replacement *Coccidioides* strains and subjecting them to phenotypic analysis.