



PROCEEDINGS OF THE 58TH ANNUAL
COCCIDIOIDOMYCOSIS STUDY GROUP MEETING

April 5, 2014

Phoenix, AZ

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COCCIDIOIDOMYCOSIS STUDY GROUP**

Meeting Number 58

April 5, 2014

University of Arizona

School of Medicine

Phoenix, Arizona

Antonino Catanzaro, M.D.

Coccidioidomycosis Study Group President

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COCCI STUDY GROUP 58TH ANNUAL MEETING

AGENDA

PHOENIX, ARIZONA

April 5, 2014

7:00-8:00 A.M. **Breakfast, Registration, Poster Set-up**

8:00-5:00 P.M. **Poster Visitation Available - Hosted in Foyer**

List of Posters by Title and Authorship

- **Pilot-Project: Attempt to Inhibit the Growth of *Coccidioides immitis* in its Natural Habitat by a Bacterial Antagonist**
Joe Baal, Derrick Chung-Sang, Abdelhamid Dalia, Julie Asato, Kiefer Rodriguez and Antje Lauer
- **Demonstration of *Coccidioides immitis* and *Coccidioides posadasii* DNA in Soil Samples Collected from Dinosaur National Monument, Utah** Suzanne M. Johnson, Erin L. Carlson, Frederick S. Fisher and Demosthenes Pappagianis
- **Detection of *Coccidioides immitis* Growth Sites in the Antelope Valley Area, California**
Yvette Sanchez, Erica Mullins, Gurleen Kaur and Antje Lauer
- **Development of a Comprehensive Rehabilitation Program for Patients Convalescing from Primary Coccidioidal Pneumonia**
Capone BL, Januszewski C, Nelson C, Galgiani JN.
- **Zero Incidence of Coccidioidomycosis in Liver Transplant Recipients after Institution of Universal Prophylaxis**
Allon Kahn, Elizabeth J Carey and Janis E Blair
- **Comparison of Nikkomycin Z Bioavailability after Single Dose Administration under Fed and Fasting Conditions**
Kathryn R. Matthias, David E. Nix, Susan Hoover, John N. Galgiani
- **Establishing a Low-Cost, High-Throughput Screening Assay for Compounds Efficacious Against the Fungus *Coccidioides*, the Causative Agent of Valley Fever**
Valentine M, Chow D, Driebe E, Bowers J, Petit J, Narang P, Meurice N, Yin H, Engelthaler D, Keim P
- ***Coccidioides* Exposures and Coccidioidomycosis Infections among Prison Employees in California**
Marie A. de Perio, Gregory A. Burr
- **Novel IL-12R β 2 Mutation Associated with Disseminated Coccidioidomycosis**
Mary E. Hanks, Amy P. Hsu, Uimook Choi, Brandon Sickle, Prabha Chandrasekaran, Un-In Wu, Christine D. Spalding, Gulbu Uzel, Alexandra F. Freeman, Harry L. Malech, Christa S. Zerbe, Steven M. Holland, Elizabeth P. Sampaio

- **The avirulent *C. posadasii* cps1 mutant has great potential as an attenuated vaccine**
Marc J. Orbach, Lisa F. Shubitz, Hema Narra, M. Alejandra Mandel and Hien Trinh
- **Coccidioides Real Time PCR using the BD MAX**
Marilyn Mitchell, Dominic Dizon, Robert Libke, Michael Peterson, David Slater, Akashdeep Dhillon
- **Development of a proteomic-based diagnostic tool for Valley Fever infection through the isolation and identification of *Coccidioides* proteins detectable in blood plasma**
Jenck, A., D. Lake, and R. U. Halden
- **Estimating the Effect of Implementing a Screening Skin Test to Reduce Valley Fever at Highly Endemic Prisons in California**
Anne E. Purfield, Kaitlin Benedict, Brian Yablon, Charlotte Wheeler, Janet Mohle-Boetani, Benjamin J. Park
- ***Coccidioides immitis* Infection in Total Knee Arthroplasty in Non-endemic Regions: a Report of the First Case in Spain**
Arbeloa, Lucas with Kuberski, Tim (by proxy)
- **California Valley Fever Network**
Erin Gaab

8:00-8:15 A.M. **Convene Meeting/Introductions/Poster Logistics/Amenities**
Antonino Catanzaro, Suzanne Johnson, Peter Kelly, Rebecca Sunenshine

8:15–8:50 A.M. **Steven Holland, M.D.**

8:15 **The Human Genetics of Disseminated Coccidioidomycosis**

8:50-9:50 A.M. **Clinical Science**
Moderator: Janis Blair

- **Pericardial Dissemination of Coccidioidomycosis, A Case Review Study**
Heidari, Arash, Johnson, Royce, Cohen, Avi, Sanchez, Negin.
- **Surgical pathology of pleural coccidioidomycosis: a clinicopathological study of 36 cases**
Tatyana A. Shekhel, Robert W. Ricciotti, Janis E. Blair, Thomas V. Colby, Richard E. Sobonya, Brandon T. Larsen
- **Assessing outdoor exposure as a risk factor for valley fever among inmates at two prisons in highly endemic areas of California**
Anne E. Purfield, Kaitlin Benedict, Brian Yablon, Charlotte Wheeler, Janet Mohle-Boetani, Benjamin J. Park
- **Disseminated Coccidioidomycosis: Risk Factors and Review of the Literature**
Camila Odio and Steven M. Holland

9:50-10:15 A.M. **Poster Visitation/Break**
Moderator: Suzanne Johnson

Posters 1-8

10:15-11:45 A.M. **Ecology and Epidemiology**
Moderator: David Engelthaler

- **Molecular detection and isolation of *Coccidioides immitis* from soil in Washington State**
Steven Hurst, Lalitha Gade, Nicola Marsden-Haug, David Engelthaler, Heather Hill, Cindy Ralston, Marcia Goldoft, Tom Chiller, Mary Brandt and Anastasia Litvintseva
 - **The Binational Project improving the Diagnosis, Surveillance, and Treatment of Coccidioidomycosis in the Border Region of “Four Corners” Arizona-Sonora and New Mexico-Chihuahua**
Dra Nubia Hernandez , Orion McCotter, Katherine Perez-Locket, Mariana Casal, Cristhian Tapia, Paul Dulin, Robert Guerrero, Dr. Gumaro Barrios, Dr Francisco Navarro Galvez, Olvera Alba Sergio, QC Rosario Aguayo, Marta Alicia Bueno, Chad Smelzer, Cesar Vera, Gloria Carrete, Ken Komatsu
 - ***Coccidioides* spp Antibodies in Domestic Dogs and Wild Rodents in Mexico**
LR Castañón-Olivares, C Segundo-Zaragoza, MR González-Martínez, MG Moctezuma-Zárate, R Muñoz-Salazar, Ana R. Montiel-Arteaga, C Toriello, A Pérez, R Laniado-Laborín, RA Cervantes-Olivares.
 - **The impact of changing laboratory reporting and testing practices on the epidemiology of coccidioidomycosis in Arizona, 2008 - 2013**
Mohammed Khan, Clarisse Tsang
 - **The Epidemiology of coccidioidomycosis in southern Arizona**
Mohammed Alzoubaidi, Orion McCotter, Catherine Golenko, and Neil M. Ampel
- 11:30 **Conducting a study on the economic burden of Valley Fever in California: results and lessons learned**
Jie Ting, Harold Lin, Paul Brown, Leslie Wilson

11:45-12:45 P.M. **Lunch**

12:45-1:00 P.M. **Dennis Dixon, Ph.D.**

12:45 **Coccidioidomycosis and the NIH: Past, Present and Future**

1:00-1:15 P.M. **Business Meeting, Cocci Study Group**
Moderator: A. Catanzaro, President, CSG

Bylaws/Voting; U.C., Merced proposal to host 59th meeting; 59th meeting date choices of March 21, 28 and April 11, 18 (Easter is Sunday, Apr. 5 and Passover is Apr. 3-11); Stewart Nielson/Skin Tests; Royce Johnson/Treasury Report; Herbert Boro/Program - duration of meeting vs. concurrent sessions/Poster presentations with abstract overload

1:15-3:00 P.M.

Laboratory and Experimental Science

Moderator: Karl Clemons

- **Whole blood cytokine patterns after *in vitro* antigen stimulation among patients with various types of coccidioidomycosis**
Chinh T. Nguyen, Lance Nesbit, Suzanne Johnson, Demosthenes Pappagianis, and Neil M. Ampel
- **Th17 immunity is essential for optimal protection against *Coccidioides* infection**
Chiung-Yu Hung, Natalia Castro-Lopez, Gary Ostroff, Tao Peng, John Galgiani, and Garry T. Cole
- **IL-8 receptor and resistance to *C. immitis* Infection**
Joshua Fierer, Suganya Viriyakosol, Lorraine Walls, Sharon Okamoto
- **A Survey of Lectin Reactivity to *Coccidioides* in Infected Human Lung Tissue**
Yasmynn Chowdhury, Setu Kaushal, Tom Grys, Janis A. Blair, Yvette Ruiz, D. Mitchell Magee, Thomas V. Colby and Douglas F. Lake
- **Screening spherule cell walls for protective vaccine antigens**
Roberts LM, Shubitz LF, Pablo J, Trinh, HT, Peng T, Aljumentas A, Sasaki R, Hermanson G, Lewis, ML, Felgner P, Davies DH, Frelinger JA, Galgiani JN
- **VT-1161, a Novel Fungal CYP51 Inhibitor, Improved Survival in a Murine Model of Coccidioidal Meningitis**
Shubitz, LF, Trinh, HT, Galgiani, JN, Lewis, ML, Garvey, EP, Hoekstra, WJ, Moore, WR, Schotzinger, RJ
- **Hybridization and Introgression among species of *Coccidioides***
Bridget Barker, Eric Lewis

3:00-3:30 P.M.

Poster Visitation/Break

Moderator: Suzanne Johnson

Posters 9-16

3:30-4:00 P.M.

Poster Forum - Questions and Answers

Moderator: Suzanne Johnson

All Poster Presenters

4:00-5:00 P.M.

Clinical Science
Moderator: Neil Ampel

- **Differentiating Lung Nodules due to Coccidioidomycosis from Lung Cancer using Clinical and Radiographic Features**
Reza Ronaghi, Ali Rashidian, Paul Mills, Michael W. Peterson
- **Serum Procalcitonin Levels in Acute Coccidioidomycosis Infections**
Kenneth K. Sakata, Thomas Grys, Yu-Hui Chang, Holenarasipur R. Vikram and Janis E. Blair
- **Multi-center Laboratory Investigation of Coccidioidomycosis EIA Reproducibility in Patients with Confirmed Disease and Controls**
Soofia Khan, MD; Rebecca Sunenshine, MD; Mike Saubolle, PhD, DABMM; Frank Ryan, Ph.D., DABCC; Arash Heidari, MD; Kelly Barbian, MT; Megan Eguchi, MPH; Orion McCotter, MPH; Kenneth Komatsu, MPH; Benjamin J Park, MD; Mike V Lancaster, PhD
- **Incidence and Severity of Coccidioidomycosis in Subjects Receiving Corticosteroids, DMARDs or Anti-TNF- α Therapy**
Mohammad Fazel, Emily McGlamery, and Neil M. Ampel

5:00 P.M.

Concluding Remarks
Antonino Catanzaro

7:00 P.M.

Dinner at Canyon Café (by reservation)
Peter Kelly and Rebecca Sunenshine

Cocci Study Group Program Committee
Herbert Boro – Director
Karl Clemons
David Engelthaler
Suzanne Johnson

Cocci Study Group Board of Directors
Neil Ampel – President-elect
Janis Blair
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Antonino Catanzaro - President
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Jessica Einstein
Rafael Laniado-Laborin
Royce Johnson
Rebecca Sunenshine

2014 Cocci Study Group Hosts
Peter Kelly
Rebecca Sunenshine

Maricopa County Department
of Public Health

Lia Koski
Jamie Wells

Arizona Department of
Health Services

Mohammed Khan
Clarisse Tsang

CSG Satellite Meeting: “Coccidioides in the Soil”

Directors: Bridget Barker and David Engelthaler
Sign-up: at CSG meeting April 5
Fee: **no fee** for registered attendees at the CSG meeting
Time: 10:00-12:00 noon on Sunday, April 6, 2014
Location: TGen HQ (next to the Phoenix Biomedical Campus)

Pilot-Project: Attempt to inhibit the growth of *Coccidioides immitis* in its natural habitat by a bacterial antagonist

Joe Baal, Derrick Chung-Sang, Abdelhamid Dalia, Julie Asato, Kiefer Rodriguez and Antje Lauer

California State University Bakersfield, Department of Biology, 9001 Stockdale Highway, Bakersfield, CA

Background: In its natural soil habitat, *Coccidioides immitis*, the causative agent of valley fever in California, likely encounters different bacterial and fungal antagonists which compete for space, water, and nutrients.

Methods: This project aimed at inhibiting the growth of the pathogen in the soil by using a strain of *Bacillus subtilis* which had shown strong anti-fungal activity against *C. immitis* *in vitro*. Three cocci-positive and three cocci-negative sites (1 m²) in an area southwest of Bakersfield were selected for a pilot-biocontrol experiment to suppress the growth of *C. immitis* in its natural habitat. All sites shared similar soil physical and chemical parameters, and the presence or absence of the pathogen was determined by using a culture independent PCR-based approach. One cocci-positive and one cocci-negative site were treated with *B. subtilis* cells/mL (~64,000 cells/mL), suspended in a saline solution (0.85 %, 1,200 mL). Another cocci-positive and a cocci-negative site were treated with sterile saline solution only. The sites were treated every two weeks from December 2012 through January 2013, for a total of four treatment sessions. The remaining cocci-positive and cocci-negative sites were left untreated to serve as control sites.

Findings: The PCR-based analyses of post-treatment soil samples suggested that our “anti-cocci” solution was not effective in inhibiting the growth of *C. immitis*. Although the pathogen was not detected in the first sampling of the *C. immitis*-positive site after the initial treatment with *B. subtilis*, the samples from this site became positive again later on during the treatment.

Conclusions: The bacterial antagonist might not work very well *in situ*, its concentration in the sprayed solution was not high enough, or did not penetrate the soil deep enough to be successful in inhibiting the pathogen. Furthermore, the cocci-negative site treated with saline solution exhibited *C. immitis* growth, which may suggest that the saline solution simulated springtime conditions that promoted growth of soil microbes including the pathogen. Also, we have to consider that some of our supposedly *C. immitis*-negative control sites might have contained the pathogen, but the amount of DNA extracted from the soil samples was under the detection limit of our PCR-based method at the start of the project.

The Epidemiology of Coccidioidomycosis – 15 California Counties, 2007-2011

Michael Mac Lean, M.D., Kings County Department of Health

Background: Fifteen California's most endemic for coccidioidomycosis (CM) counties provided epidemiology data on cases reported for the period 2007-2011. The data identified institutional cases, primarily state and federal prisoners. California data hasn't previously been reported by institutional status of cases.

Findings: During the five year interval the 15 counties reported 16,843 cases. Nineteen percent of the reported cases were institutional. In many of the counties the observed incidence rate increased significantly after 2009. The increase was noted only in the non-institutional, community cases. In ten of the fifteen counties the risk for CM wasn't evenly distributed by residence of reported cases. All of California's major north-south highways traverse highly endemic areas. Kern County had the highest observed mean incidence rate followed by other southern San Joaquin Valley counties. The number of cases reported outside the southern SJV was significant. The high incidence rate in male inmates skewed the gender distribution of the cases in the 15 counties; 66% of the cases were male. After adjusting for inmate cases, the 59% male cases in the 15 counties remained significantly higher than is seen in Arizona. Although the data did not include the age distribution of the inmate cases, the high number of inmate cases very likely skewed the age distribution of the cases. The data collected in the 15 counties is too incomplete to permit any reasonable estimate of disease acquisition by race/ethnicity. Because some R/E groups are under-represented in the populations of the most endemic areas, reliable estimates of risk by R/E may remain elusive even with more complete collection of R/E data by the counties. Thirteen of the counties reported one or more institutional cases; in two counties the inmate cases represented a majority of the cases.

Conclusions: The observed institutional rates consistently and significantly exceeded each county's observed community incidence rate. Even when compared with adjacent communities, the observed rate in inmates remained significantly elevated. Four or more of the prisons had mean rates rarely exceeded elsewhere. The less endemic counties in California are over-represented in California hospitalization data.

Demonstration of *Coccidioides immitis* and *Coccidioides posadasii* DNA in soil samples collected from Dinosaur National Monument, Utah

Suzanne M. Johnson^a, Erin L. Carlson^a, Frederick S. Fisher^b, and Demosthenes Pappagianis^a

Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis, and Department of Geosciences, University of Arizona, Tucson^b

Background: In 2006, soil samples were collected from Dinosaur National Monument (DNM), Utah, the site of a coccidioidomycosis outbreak. Preliminary evaluation of these soils included injection of soil extracts into Balb/c mice. Spherules were observed in direct mounts of tissue collected following necropsy of mice injected with soil extracts from SS06RH and SS06UM indicating that soil samples contained viable *Coccidioides*. Both soils were composite samples collected in the midden area near the Swelter Shelter. SS06RH was obtained from a rodent hole near the midden base while SS06UM was from the upper midden.

Methods: Recently, DNA was isolated from these two soil samples, and a nested endpoint PCR was used to amplify *Coccidioides* DNA present in the samples. Products representing the ribosomal RNA genes and internal transcribed spacer (ITS) region were obtained and sequenced. Previous studies have shown that single-nucleotide polymorphisms present in ITS 1 and ITS2 region could be used to assign the appropriate *Coccidioides* species. Sequence analysis of the PCR products indicated that the *Coccidioides* DNA amplified from sample SS06RH was that of *Coccidioides immitis*, while that from sample SS06UM was *Coccidioides posadasii*.

Conclusion: This is the first report of direct demonstration of *Coccidioides* in soils from Dinosaur National Monument and the first report of the presence of both *C. immitis* and *C. posadasii* from the same geographic location.

Detection of *Coccidioides immitis* growth sites in the Antelope Valley Area, California

Yvette Sanchez, Erica Mullins, Gurleen Kaur and Antje Lauer

California State University Bakersfield, Department of Biology, 9001 Stockdale Highway, Bakersfield, CA

Background: Coccidioidomycosis incidence has increased significantly in the Antelope Valley Area (Los Angeles County, CA) and Kern County, since the late 1990's.

Methods and Findings: We investigated 110 soil samples from locations near Edwards Airforce Base (EAFB), and 75 soil samples from around Lancaster and Palmdale for the presence of *C. immitis* with a nested PCR approach that included a diagnostic PCR step using primers that target the intertranscribed spacer region ITS 1 of the ribosomal gene. DNA was extracted from all samples and the presence of fungal DNA was confirmed for almost all samples. We found that four soil samples from two sites close to the Loma Vista Detention Center near Lancaster/Quartz Hill contained the pathogen, in addition to several sites near the EAFB that supported the growth of the pathogen. To confirm that the obtained PCR products indeed indicated the presence of *Coccidioides* spp., all PCR products of the expected size were sequenced and compared to entries of spp. in the nucleotide database of the National Center of Bioinformatics (NCBI).

Next Steps: Our next step is to correlate the presence/absence of the pathogen to certain soil parameters, such as pH, organic matter, and soil particle sizes (% of silt, sand, clay).

Development of a comprehensive rehabilitation program for patients convalescing from primary coccidioidal pneumonia.

Capone BL¹, Januszewski C¹, Nelson C¹, Galgiani JN².

¹Ambulatory Rehabilitation Department, St. Joseph's Hospital and Medical Center, and

²Valley Fever Center in Phoenix, Phoenix Arizona.

Background: Patient's with primary coccidioidal pneumonia frequently experience a fatigue syndrome extending weeks to occasionally many months beyond resolution of other signs and symptoms of the infection. To what extent this protracted fatigue is a symptom of active disease or the consequence of the resulting deconditioning is unknown. Although comprehensive rehabilitation has been effective in managing patients with fibromyalgia, with chronic pain and after lung transplantation, an analogous program does not exist for addressing valley fever fatigue.

Findings: We have developed a prototype reconditioning program that includes baseline and serial reassessments of strength and endurance. Short-term and long-term goals were established by each patient at the onset of therapy. Since 2012, 20 patients with a definite or presumed diagnosis of coccidioidomycosis have begun our program. Nearly all had primary, uncomplicated pneumonia. Outpatient visits were one or two times weekly and were completed between 4 and 6 weeks on average. Self reported improvement in overall functional levels was on average 50 points on a 100 point scale.

Conclusions: Though objective improvements to strength and endurance have been demonstrated with all patients the data has not yet been formally analyzed. Our experience to date encourages us to expand our program and may be useful to other clinicians who care for patients with this problem.

Zero Incidence of Coccidioidomycosis in Liver Transplant Recipients after Institution of Universal Prophylaxis

Allon Kahn, M.D.¹, Elizabeth J Carey, M.D.¹ and Janis E Blair, M.D.¹

¹Mayo Clinic, Scottsdale, AZ, United States.

Introduction: We previously identified a 3.1% incidence of de novo and recurrent coccidioidomycosis (cocci) in liver transplant (LT) recipients despite targeted antifungal prophylaxis. This led to the implementation of a universal prophylaxis program in February 2011. We compared cocci incidence rates between targeted and universal prophylaxis cohorts, hypothesizing that the expanded prophylaxis program would decrease the incidence.

Methods: Retrospective review of all LT recipients between February 4th, 2011 (initiation of universal prophylaxis) and July 11th, 2013. The prophylactic antifungal regimen consisted of fluconazole 200 mg daily for the first post-LT year, adjusted for kidney function. Exclusion criteria were: retransplant, dual organ transplant, death in the first post-LT year, and lack of 12 month follow-up. Data from the universal prophylaxis cohort were compared to previously published data from the targeted prophylaxis era. One-year cocci incidence was compared between groups for all endemic patients.

Results: Of the 160 LT recipients during the study period, 108 met inclusion criteria. 65% completed 12 months of full-dose prophylaxis. 22% required dose adjustment for kidney function. 4% of patients permanently discontinued prophylaxis for adverse events (elevated LFT, pancytopenia), while 3% required temporary cessation or dose reduction. Compared to the 349 patients in the prior study, the universal prophylaxis cohort was significantly older with a higher prevalence of HCC, renal insufficiency, and post-LT diabetes mellitus. They also exhibited significantly higher rates of pre-LT cocci and asymptomatic seropositivity. They were equally likely to experience acute cellular rejection, however less likely to receive IV steroid therapy if it occurred. Among the 90 endemic patients, no cases of cocci were observed. Compared to the prior study, in which 10 of 325 (3.1%) endemic patients experienced post-LT infection, this difference did not reach statistical significance ($P = 0.127$).

Conclusions: No cases of cocci were observed in the universal prophylaxis cohort despite a higher pre-LT history of infection, older age, and higher prevalence of diabetes and asymptomatic seropositivity. The lack of statistical significance between groups is attributed to the limited statistical power imposed by small sample size. The fluconazole regimen was well-tolerated with few adverse events. These data support the use of a universal fungal prophylaxis program in endemic regions.

Comparison of Nikkomycin Z Bioavailability after Single Dose Administration under Fed and Fasting Conditions

Kathryn R. Matthias, PharmD; David E. Nix, PharmD; Susan Hoover, MD, PhD, John N. Galgiani, MD

Background: Nikkomycin Z, a chitin synthase inhibitor, has activity against *Coccidioides* species. This pilot study was conducted to evaluate the relative bioavailability of nikkomycin Z in healthy subjects following administration of a single dose with a high fat meal compared to the same dose administered under fasting conditions.

Methods: Four healthy adult subjects received nikkomycin Z in this pilot open label, single dose, cross-over study. Each subject received nikkomycin Z 500 mg after an overnight fast or 15 minutes after a high-fat, high-calorie meal. The standardized meal consisted of approximately 150 calories from protein, 250 calories from carbohydrates, and 500 calories from fat. Blood and urine sampling occurred at several time points and measured concentrations were evaluated using a non-compartmental analysis.

Results: In fed subjects, the mean \pm standard deviation maximum concentration was higher at 8.22 ± 1.54 mcg/mL than in fasting subjects at 5.20 ± 1.32 mcg/mL. The median time to maximum concentration was 2 h later in fed subjects compared to fasting subjects. High fat food intake increased bioavailability with increases in mean AUC_{0-24} and $AUC_{0-\infty}$ of 215% and 229%, respectively compared to fasting condition.

Conclusion: High-fat, high-calorie meals significantly altered the increased bioavailability of nikkomycin Z following oral administration in healthy adults.

Establishing a Low-Cost, High-Throughput Screening Assay for Compounds Efficacious Against the Fungus *Coccidioides*, the Causative Agent of Valley Fever.

Valentine M¹, Chow D², Driebe E¹, Bowers J¹, Petit J³, Narang P³, Meurice N³, Yin H², Engelthaler D¹, Keim P¹

¹Translational Genomics Research Institute, Center for Public Health and Clinical Pathogens, Flagstaff, AZ

²Translational Genomics Research Institute, Cellular Genomics Collaborative Center, Cancer and Cell Biology Division, Scottsdale, AZ

³Mayo Clinic, Department of Research, Scottsdale, AZ

Background: The inhalation of airborne spores of the endemic soil fungus *Coccidioides* can cause Valley Fever. For people suffering Valley Fever, treatment options are limited and drug discovery pipelines for the disease are scant, as the significant costs to discover and deliver new treatments are deterrents. Bringing together the microbiology expertise of TGen North, the compound management, chemoinformatics, and medicinal chemistry expertise of the Mayo Clinic, and the expert chemical compound high throughput screening of the Cellular Genomics Collaborative Center (CGCC) within the TGen Division of Cancer and Cell Biology, we have developed a low cost, high-throughput, prototype, screening assay for the discovery of compounds efficacious against *Coccidioides*.

Findings: Our pipeline has been validated in 96-well plate format using manual pipetting, 384-well plate format using automated liquid handling, and makes use of microtiter plate spectrophotometry (OD 600nm) to determine compound inhibitory effects on the growth of the organism. To establish a pipeline, we screened the well characterized Prestwick and LOPAC small molecule libraries in a biosafety level-2 laboratory (BSL-2) setting, using the avirulent strain *C. posadasii* Δ cts2/ Δ ard1/ Δ cts3. In the 96-well format, 70 hits were identified from a collection of 1655 compounds consisting of 1200 in the Prestwick library and 455 knowledge-driven compounds. The 70 identified compounds inhibited growth >90% at a concentration of 20 μ M. In order to increase the throughput for a HTS environment, we established a screening protocol for working in a 384-well format using the avirulent strain. We performed replicate screens of the 1,280 member LOPAC at final concentrations of 1 and 5 μ M. Hits were selected if they inhibited growth >50% at any time point and concentration in both runs. Our data showed 35 compounds were prioritized as hits from LOPAC library screen, representing a hit rate of 2.7%.

Conclusions: This process is a first step towards a protocol workable in a BSL-3 laboratory allowing for the screening of virulent *Coccidioides* or for screening other BSL-2 fungi. The ability to screen thousands of compounds quickly and inexpensively could result in more therapeutic options for patients with Valley Fever.

***Coccidioides* Exposures and Coccidioidomycosis Infections among Prison Employees in California**

Marie A. de Perio, Gregory A. Burr

Background: Coccidioidomycosis, also known as valley fever, is an illness caused by inhalation of spores of the fungus *Coccidioides*, which grows in the soil in the southwestern United States. In April 2013, employer representatives requested a health hazard evaluation by the Centers for Disease Control and Prevention's National Institute for Occupational Safety and Health concerning employee exposure to *Coccidioides* at two state prisons in central California. As part of this evaluation, we determined the incidence of coccidioidomycosis among employees and assessed ways to minimize exposures at the prisons.

Methods: We determined crude incidence of coccidioidomycosis among employees from 2009–2013 using annual employee rosters and the California Department of Public Health coccidioidomycosis database. During visits to the prisons in June 2013, we interviewed a convenience sample of 172 employees about their work practices and exposures, assessed the ventilation in selected buildings, and met with prison staff to learn about mitigation efforts.

Results: We identified 65 confirmed cases of coccidioidomycosis among prison A employees and 38 confirmed cases among prison B employees from 2009–mid-2013. These were reported by nine California counties of residence. The crude average annual incidence was 1,039 cases per 100,000 employees for prison A and 511 cases per 100,000 employees for prison B over this time. Interviews with employees revealed that they may be potentially exposed to *Coccidioides* in the outdoor and indoor work environment and outside of work.

Conclusions: Both prisons are located in hyperendemic areas for coccidioidomycosis, and prison employees are exposed to *Coccidioides* both at work and outside of work. The crude average annual incidence among employees appears to be higher than that reported among the general adult population in the surrounding counties. Measures such as wetting soil before disturbing, re-vegetating prison grounds, stabilizing soil, paving roads, and improving building ventilation would be expected to reduce dust exposures to varying degrees. However, none of these measures will eliminate exposure to *Coccidioides*, and their relative effectiveness in reducing occupational coccidioidomycosis is unknown.

Novel IL-12R β 2 Mutation Associated with Disseminated Coccidioidomycosis

Mary E. Hanks¹, Amy P. Hsu¹, Uimook Choi², Brandon Sickle¹, Prabha Chandrasekaran¹, Un-In Wu¹, Christine D. Spalding¹, Gulbu Uzel¹, Alexandra F. Freeman¹, Harry L. Malech², Christa S. Zerbe¹, Steven M. Holland¹, Elizabeth P. Sampaio¹

¹Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, MD; ²Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, MD.

Background: Disseminated coccidioidomycosis has been linked to mutations in IFN- γ R1, IL-12R β 1, IL-12p40, STAT1, and STAT3 genes. Signaling through the IL-12 receptor leads to the proliferation and differentiation of NK and Th1 cells into IFN- γ secreting cells.

Findings: A 27 year old man presented with severe disseminated coccidioidomycosis (*Coccidioides immitis*) with progressive bony lesions and pulmonary abscesses. Whole exome sequencing identified a novel heterozygous mutation in the IL-12-beta 2 receptor (c.302 G>A, p.C101Y), predicted to be deleterious. Patient PHA/IL-2 stimulated T cell blasts showed normal membrane expression of the IL-12R β 2 protein but did not normally phosphorylate STAT4 in response to IL-12 (50-300ng/mL), while healthy controls did. Decreased production of IFN- γ and TNF- α were detected in patient's PBMC following stimulation with IL-12, but not PHA or PHA+IL-12.

Conclusions: This is the first description of a mutation on IL-12R β 2 associated with fungal disease. The IL-12/IFN- γ pathway is critical for the control of *Coccidioides*.

The avirulent *C. posadasii* cps1 mutant has great potential as an attenuated vaccine

Marc J. Orbach, Lisa F. Shubitz, Hema Narra, M. Alejandra Mandel and Hien Trinh

Background: One approach to identify *Coccidioides* virulence factors, which may be targets for therapeutics, is to disrupt genes that are important for pathogenicity in plant pathogens and/or other animal pathogens.

Methods: Based on the work of Liu et al. (2003), we disrupted the *Coccidioides* ortholog of *C. heterostrophus* CPS1 in *C. posadasii* strain Silveira. CPS1 was originally identified as a potential non-ribosomal peptide synthase (NRPS) component, based on the presence of two AMP binding domains related to the adenylation domains in bacterial non-ribosomal peptide synthases. However it also contains a putative N-terminal DMAP1b domain, named because in mammals it binds the DMAP1 transcriptional co-repressor. Thus this gene may play a regulatory role in *Coccidioides*. Deletion of the *C. posadasii* CPS1 gene results in a strain that is non-pathogenic in susceptible mice but does initiate the formation of spherules, both in vivo and in vitro. The cps1 mutant appears to have great potential as an attenuated vaccine since it protects mice from further infection.

Findings: When susceptible C57BL/6 or BALB/c mice were challenged with wild type *C. posadasii* after vaccination with the cps1 mutant, nearly all experienced extended survival of at least four weeks and had low fungal burdens. The safety of this potential vaccine was demonstrated by the avirulence of the cps1 mutant upon infection of highly immunodeficient NOD-scid, γ c^{null} (NSG) mice.

Conclusions: Whether CPS1 plays a role as a regulator of virulence via the DMAP1b domain, or via production of a potential NRPS toxin is not known. This is being explored via RNA-seq analysis and isolation of secreted metabolites from both the wild type strain Silveira and the cps1 mutant.

Reference: Lu, S. W., S. Kroken, B. N. Lee, B. Robbertse, A. C. L. Churchill, O. C. Yoder, and B. G. Turgeon. 2003. A novel class of gene controlling virulence in plant pathogenic ascomycete fungi. *Proceedings of the National Academy of Sciences of the United States of America* 100:5980-5985.

Coccidioides Real Time PCR using the BD MAX

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Background: Rapid Real Time polymerase chain reaction (RT-PCR) can be performed in a community hospital setting using the new Becton Dickinson molecular instrument, the BD MAX.

Findings: Following sample preparation, both the DNA extraction and the PCR were performed on the BD MAX using the BD MAX ExK DNA-1 extraction test strip and a Master Mix prepared by BioGX (Alabama). Sample preparation took two (2) hours and testing on the BD MAX took an additional two (2) hours. Method sensitivity and specificity were evaluated along with the limits of detection to confirm that this convenient method would provide medically useful information. Using serial dilutions, the lower limit of detection was determined to be 1 cfu/ul . Testing with this method was validated using various body sites including bronchial alveolar lavage (BAL), sputum, lung tissue, pleural fluid and spinal fluid.

Safety protocols were established and specimen preparation processes were developed for the various types of specimens. The Ct range which signified a positive result was established along with the acceptable internal standard Ct value range. Positive controls run with each batch were prepared by spiking a pooled BAL specimen with a known dilution of *Coccidioides immitis* organism.

Conclusions: Our experience testing over 200 samples shows clinically relevant information can be available within 5 hours using a RT-PCR method on the BD MAX to identify *Coccidioides immitis* in a variety of specimen types with sensitivity equivalent to culture.

Development of a proteomic-based diagnostic tool for Valley Fever infection through the isolation and identification of *Coccidioides* proteins in detectable in blood plasma

Jenck, A., D. Lake, and R. U. Halden

Background: Coccidioidomycosis, more commonly known as Valley Fever, is a disease endemic to the southwestern United States. It is not known what components of the fungus circulate in blood from endospores and spherules growing in the lung. In an attempt to define protein components of *Coccidioides* in the blood of those infected, this ongoing study aims to extract and isolate the *Coccidioides* proteins from the plasma of infected subjects and identify them via mass spectrometry.

Method: Plasma samples of infected and healthy patients are purified by affinity chromatography using a lectin that has been shown to bind to spherules and endospores in infected lung tissues. Fungal proteins are eluted from the lectin, separated, and trypsin-digested for qualitative analysis by matrix-assisted laser desorption ionization tandem time-of-flight mass spectrometry (MALDI TOF-TOF MS).

Results: Results obtained thus far provide mass spectrometric evidence for the ability to extract and identify *Coccidioides* proteins in blood plasma using this method. This study is one of multiple steps required to bring us closer to a rapid, accurate method for diagnosing coccidioidomycosis enabled here by mass spectrometry-based proteomics.

Estimating the Effect of Implementing a Screening Skin Test to Reduce Valley Fever at Highly Endemic Prisons in California

Anne E. Purfield, Kaitlin Benedict, Gordana Derado, Janet Mohle-Boetani, Charlotte Wheeler, Benjamin J. Park

Background: Valley Fever (VF) is a respiratory disease caused by inhalation of *Coccidioides* spp. spores; prior infection confers lifelong immunity. During 2011, VF incidence at two highly endemic prisons (HEP) was >1000% higher than surrounding areas. Screening inmates with a new delayed-type hypersensitivity skin test (Spherusol®) that identifies persons with prior *Coccidioides* infection could be used to populate HEP with immune inmates. We developed a model to estimate change in VF incidence following implementation of a Spherusol® screening program.

Methods: In this screening program, Spherusol®-negative (non-immune) inmates are excluded from HEP; prisons are repopulated with Spherusol®-positive inmates. Data from prison clinics on laboratory-confirmed infections were used to calculate incidence rates, using 2011 mid-year population at HEP. We calculated the likelihood of prior infection among HEP inmates based on county of inmate prosecution because infection rates vary geographically, and used published estimates of Spherusol® sensitivity and specificity. A sensitivity analysis varied prevalence of prior infection and test sensitivity and specificity.

Results: Without a Spherusol® screening program, we expect an annual incidence of 5.3% at HEP. According to the model, 87% of inmates will test negative and will be excluded from HEP. Following re-population of HEP with Spherusol®-positive inmates, the expected incidence will be 1.8% (65% decline, 284 cases prevented); inmates with false-positive Spherusol® tests will comprise 99% of subsequent infections. Sensitivity analysis demonstrated an expected incidence of 0.6-4.4% (17-88% decline; 77-387 cases prevented), largely influenced by prior infection prevalence and test specificity.

Conclusion: Implementation of a hypothetical Spherusol®-based screening could sharply reduce incidence of VF at HEP. If implemented, additional studies to refine assumptive values and evaluate the effectiveness of a screening program should be considered.

Key Words: Valley fever, coccidioidomycosis, prison, modeling

***Coccidioides immitis* infection in Total Knee Arthroplasty in Non-endemic regions.
Apropos of the first case in Spain**

Lucas Arbeloa
with Tim Kuberski (by proxy)

Background: Fungal infections of joint prostheses are very rare and are generally difficult to treat. Today it is not clear how it should be treated and how long to maintain the treatment, whether surgical cleaning is required and whether or not required implant removal. There is more controversial and less evidence if it fits in the treatment of fungal infections of *Coccidioides* species, an endemic fungus of the Southwest of North and Central America, which is spread by spores infecting and causing airway clinic, "*Valley Fever*", only 40 % of those infected. Generally these symptoms are indistinguishable from a respiratory infection in the upper airways, but in less than 1 % of cases a systemic dissemination occurs. In these cases bones and joints are involved in about 20 to 50% of the times. In patients with prosthetic joints, hematogenous spread of this fungus can produce late infections, which are difficult to diagnose and with a complicated handling and treatment, especially when they occur in non-endemic regions. The differential diagnosis is difficult, delaying the beginning of appropriate treatment and worsening the prognosis of the implant. The classic treatment was based on prolonged use of Amphotericin B, which was replaced in recent years by the Azoles (Fluconazole, Itraconazole...), due to fewer side effects with comparable efficacy, being relegated to special cases of intolerance or resistance to azoles or during pregnancy.

Findings and Conclusion: We report the first case of prosthetic joint infection by the fungus *Coccidioides immitis* in Spain and reflect on the difficulty of diagnosis and treatment of this prosthetic infection in non-endemic regions.

California Valley Fever Network

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Background: While coccidioidomycosis has long been a problem in California's San Joaquin Valley, it has only recently received increased attention from policy makers and the media.

Findings: The increased attention has led to researchers in the region coming together to identify the questions that remain unanswered regarding Valley Fever in San Joaquin Valley. Two meetings were held with researchers, clinicians, and community groups from the region, with over 70 participants. At the first meeting, researchers identified known and unknown information about coccidioidomycosis in the San Joaquin Valley. At the second meeting, community groups and policy makers joined the researchers to develop priorities for coccidioidomycosis research in the region. The meetings focused on four areas: Biomedical questions including the basic science of the fungus and disease, clinical concerns around current practice for detecting and treating coccidioidomycosis in the San Joaquin Valley, public health considerations regarding the best strategies for prevention and detection of coccidioidomycosis, and participatory action research to increase our understanding of the patient experience and how to advocate for more resources to be directed toward this area. The purpose of this presentation is report on the outcomes from these meetings, summarizing the conclusions from each area.

Conclusions: Taking lessons learned from other infectious disease prevention and detection campaigns, we review what is known about reducing the effects of the condition on the community. It is our hope that the discussion will stimulate a wider dialogue with experts in Valley Fever to improve our coordination of Valley Fever actions in California.

Pericardial Dissemination of Coccidioidomycosis, A Case Review Study

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Background: Extrapulmonary manifestation of Coccidioidomycosis has been reported to have an incidence of 4.7% of all recognized infections and 0.2% of all respiratory exposures. Among these extrapulmonary manifestations the cardiac manifestations especially pericardial dissemination is not well reported in the literature. Some sources report 1-5% of infected patients will suffer from pericarditis. We would like to bring to the attention of medical community 5 cases of pericardial involvement of Coccidioidomycosis at our center and our experience in treating these patients.

Results: Our patient population was noted to be 80% African American male & 20% Hispanic male with mean age of 29.6 and median of 23 years old at the time of diagnosis. 4 out 5 denied history of IV drug use but history of 1 case was unknown. Range of Cocci Serology levels were noted to be minimum of 1/32 and maximum of >1/512 with maximum peak of >1/512 and minimum peak of 1/128. Median peak value was noted to be 1/256. 25% (1/5) of cases had positive Cocci culture vs. 4/5 cultures were negative. Site of infection varied among the 5 cases but they all had at least pulmonary and pericardial involvement with 100% pericardial effusion, 65% showed pericardial thickening, cardiomegaly was noted in 45% of the cases, 4 cases that had gotten ECG showed sinus tachycardia in 100% of the cases, none of the patients had received a TEE, but 4 cases had TTE done with results confirming pericardial effusion in 75% of the cases. 2/5 cases are known to have undergone pericardectomy. 4 cases had bone scan done and results showed 50% radiotracer activity.

Conclusion: Coccidioidomycosis involvement of pericardium is a rare occurrence, and only twenty cases have been described in the literature. Studies have shown some ethnic groups such as Filipinos and African Americans are more susceptible to severe disseminated disease. Our data from a hospital located in an endemic region of the US shows that the majority of patients affected were African American male. Coccidioidomycosis is a great imitator and can be mistaken for tuberculosis and other granulomatous diseases. A history of patient indicating exposure to this fungus in an endemic area along with positive serologic test and positive cultures from relevant sites are some of the valuable diagnostic tools available to clinicians to make the diagnosis and start appropriate therapy. In case of pericardial involvement of Coccidioidomycosis the most appropriate therapy is administration of Amphotericin B 0.5-0.7 mg/kg/day IV for six weeks. In cases of renal insufficiency a lipophilic formulation of this drug may be considered as an alternative.

Surgical Pathology of Pleural Coccidioidomycosis: a Clinicopathological Study of Thirty-six cases

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Background: Most pulmonary coccidioidal infections are intraparenchymal; the pleurae are rarely involved. Pleuritis is a recognized complication of ruptured cavitory infections and occasionally occurs in other settings, but this has not been fully characterized.

Methods: To define the clinical and histopathologic characteristics of pleural coccidioidomycosis as encountered by surgical pathologists, the clinical history, imaging, and histology of 36 biopsy-, resection-, or autopsy-confirmed cases (with coccidioidal spherules present in pleural tissue; median age 39 yrs; 22 men) were reviewed. These represented 7% of all pulmonary coccidioidal infections, and showed two modes of presentation, including ruptured cavitory infection (26) and pleural-predominant disease with milder parenchymal involvement (10). Risk factors included immunodeficiency, smoking, and occupational exposure to soil. Common symptoms (median 5 weeks) included cough (47%), chest pain (44%), and dyspnea (39%). Imaging often showed pleural adhesions (64%) and effusions (61%). Treatment included lobectomy or decortication, with antifungal medications.

Findings: All cases showed granulomatous pleuritis. Both modes of presentation showed similar histologic features, including the composition of inflammatory infiltrates, degree of fibrosis and extent of necrosis. Spherules were usually few (mean density <1/10 hpf). Three deaths occurred (all with ruptured cavities); the remaining patients recovered.

Conclusions: The differential diagnosis of pleural effusions should include coccidioidomycosis, particularly in endemic areas, even without significant intrapulmonary disease. Most cases of coccidioidomycotic pleuritis are encountered by pathologists after resection of ruptured cavities with decortication, but pleural predominant infections may be biopsied for diagnostic purposes. Spherules are usually rare in pleural tissue, and liberal sampling, cultures, or serologic studies may be required to confirm the diagnosis.

Assessing Outdoor Exposure as a Risk Factor for Valley fever Among Inmates at Two Prisons in Highly Endemic Areas of California

Anne E. Purfield, Kaitlin Benedict, Brian Yablon, Charlotte Wheeler, Janet Mohle-Boetani, Benjamin J. Park

Background: During 2011, Valley fever incidence in highly endemic areas at two California state prisons, Avenal State Prison (ASP) and Pleasant Valley State Prison (PVSP), was >1000% higher than the surrounding community. We performed a matched case-control study to assess if type or duration of outdoor exposures were associated with illness among inmates at ASP and PVSP.

Methods: A case was defined as Valley fever diagnosed in an inmate at ASP or PVSP who spent at least one night at the prison in 2011; controls were inmates not diagnosed with Valley fever during the same time period and were matched by age, race, and prison yard of residence. We used a standardized questionnaire to interview cases and a similar questionnaire for controls.

Results: We identified 40 cases and 174 matched controls. Thirty-four (85%) cases and 147 (85%) controls reported frequently breathing in dirt or dust at the prison. The most common site of dirt or dust inhalation was the outdoor prison yard (68% in cases, 62% in controls, $p=0.486$). Case-inmates spent a median of 15.9 hours (range, 0-45.5) per week outdoors, compared to 15.4 hours (range, 0-77) for controls ($p=.809$). There was no difference in the frequency of reported yard activities, which include basketball, handball, softball/baseball, running/walking, calisthenics/weight lifting, soccer, or sitting around. In addition, no difference was detected between inmate-cases and controls for prison occupation or education program.

Conclusions: We did not find that type of activity or duration of time spent outdoors was associated with Valley fever at ASP or PVSP. Limiting or modifying inmate activities outdoors may not substantially reduce Valley fever illness in these prisons.

Disseminated Coccidioidomycosis: Risk Factors and Review of the Literature

Camila Odio, B.A. and Steven M. Holland, M.D.

Background: Of the 150,000 coccidioidomycosis (CM) cases reported annually, 60% are asymptomatic, 40% are pulmonary, and <1% involve dissemination to at least two extrapulmonary organs. To better understand the risk factors for and mechanisms of severe CM, we reviewed 344 published cases of disseminated CM between 1975 and 2014.

Methods-Findings: We compared disease presentation and mortality in patients with widely disseminated infection, monodisseminated infection, immune suppression, and pregnancy. We found the highest survival rates in patients with monodissemination. Additionally, we found robust associations between race, frequency and localization of dissemination, consistent with the idea that host factors underlie the development of dissemination. Specifically, among otherwise immune competent patients, self-identified blacks had a 2 fold higher risk of disseminated disease and a 2 fold higher rate of osteomyelitis than self-identified whites. In contrast, whites had an approximately 5 fold greater risk of CNS CM than blacks. However, among patients with immune suppression, blacks had a lower risk of dissemination than whites and no significant differences were observed in disease localization.

Conclusions: Thus, subtle variations in host defenses associated with race may be overridden by the major immune suppression related to pregnancy, drugs or HIV. These differences in host defense likely involve inherited changes in the immune signaling pathways that are central to protection against *Coccidioides*. Genetic screening of seven patients with disseminated CM (Table 1) indicates that the IL-12/IFN- γ axis is critical for defense against this fungus. Patients with disseminated CM should be screened for defects in the IL-12/IFN- γ axis.

Table 1. Patients with disseminated coccidioidomycosis and discrete defects in the IL-12/IFN- γ axis

No.	Pulmonary disease	Extra-pulmonary Disease	Genetic Findings	Initial/ Final Therapy	Final Outcome
1	+	Meningitis	STAT3 het. (c.2137G>A)	A/ F	Controlled with lifelong fluconazole
2 ¹	+	Meningitis and cerebral abscess	STAT3 het. (A>T in exon 14, p.T412S)	A and F/ F	Controlled with lifelong fluconazole

3 ^{2*}	+	Osteomyelitis erythema nodosum, cutaneous lesions	IFN- γ R1 def. (818del4)	A/ IFN- γ	Clinical improvement
4 ³	NR	Diffuse lymphadenitis	IL-12R β 1 hom. (c.557G>A, p.C186Y)	F	Clinical resolution
5 ³	+	Osteomyelitis, lymphadenitis, nasal lesion,	IL-12R β 1 hom. (c.557G>A, p.C186Y)	F/ I	Clinical improvement
6 ⁴	+	Osteomyelitis, soft tissue, cutaneous lesions	STAT1 GOF (c.1057G>A, p.E353K)	A and V/ P	Poorly controlled and persistent CM
7 ⁴	+	Osteomyelitis, bilateral cerebral lesions, intrathoracic lymphadenitis	STAT1 GOF (c.800C>T, p.A267V)	F/ C,V and P	Died of CM at 17 y
8	+	Osteomyelitis, soft tissue	IL-12R β 2 het. (c.302G>A, p.C101Y)	A and D/ IL-12, IFN- γ , P	Poorly controlled and persistent CM

A: Amphotericin, C: Caspofungin, D: Diflucan, F: Fluconazole, I: Itraconazole, P: Posaconazole, V: Voriconazole

*Patients 3 and 4 are siblings and their parents are first cousins.

[†] HIES: hyperimmunoglobulin E (Job's) syndrome

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Molecular detection and isolation of *Coccidioides immitis* from soil in Washington State

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Background: *Coccidioides immitis* is a soil-dwelling fungus, which is endemic to the southwestern US. Until recently, *Coccidioides* sp. had never been documented in Washington State. A recent report by Marsden-Haug et al describes three cases of acute coccidioidomycosis in patients from Washington State, whose epidemiological and clinical data suggest local acquisition of the infection.

Methods: To evaluate presence of *Coccidioides* sp. in Washington soils, we obtained 22 soil samples collected in locations thought to be plausible sources of the patients' infections based on epidemiologic information. We used novel real-time PCR assay developed by Translational Genomics Institute to detect presence of fungal DNA in soil. We assessed specificity of this real-time PCR assay by testing DNA extracted from the non-endemic soils and other related fungi. To isolate viable strains of *Coccidioides* from soils, we used previously described yeast extract medium with modifications.

Results: Real-time PCR was specific and produced positive results with DNA extracted from *C. immitis* and *C. posadasii* cultures and confirmed *Coccidioides*-positive soils; negative results were obtained with all negative control soils and cultures. Six of 22 soil samples from two sampling sites were positive by the real-time PCR. Four of these soil samples were also positive by culture. Fourteen strains of *C. immitis* were isolated and confirmed by sequencing of rDNA and three other protein-coding genes. Genotypes of the environmental strains and their relationships with a clinical isolate from one of the WA patients are now being evaluated.

Conclusions: Our data provide the first molecular and microbiological confirmation of *C. immitis* in soils of Washington State.

The Binational Project improving the Diagnosis, Surveillance, and Treatment of Coccidioidomycosis in the Border Region of “Four Corners” Arizona-Sonora and New Mexico-Chihuahua

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3. New Mexico Department of Health
4. Chihuahua State Health Services

Introduction: Coccidioidomycosis (Valley fever, cocci) is endemic along much of the US-Mexico Border Region. The public health implications of Valley Fever are a concern to the border region. The clinical presentation of primary pulmonary coccidioidomycosis (cocci) is a non-descript syndrome mimicking influenza-like illness and/or tuberculosis. In Mexico, public health only receives limited reports of the disease due to the lack of a systematic and sustainable surveillance system currently for coccidioidomycosis.

Methods: Laboratory capacity training took place at InDRE (Institute of Diagnostics and Epidemiologic Reference) in Mexico City, MX offered by CDC/Mycotics Branch, and with participation of LESP (Public Health State Laboratory) staff from Sonora and Chihuahua Laboratory. The states of Sonora and Chihuahua began a pilot study to examine the undiagnosed burden of coccidioidomycosis among the already established Tuberculosis surveillance system.

Results: Since 2012, Sonora Health Secretary has collect 126 samples for testing for coccidioidomycosis by EIA. All specimens collected came from patients with respiratory symptoms and have TB smear results available. Preliminary results demonstrate that 21 (17%) are positive by EIA screen. Among the EIA positive cases 19% were only IgM reactive, 52% were only IgG reactive, and 38% were both IgM and IgG reactive.

Conclusions: The project of coccidioidomycosis in the “Four Corners” project was initiated to enhance binational surveillance of respiratory disease through leverage of existing resources and experiences in the region, as well as the implementation of and transfer of new technology in the diagnosis of the disease. These preliminary data may show an important unrecognized proportion of coccidioidomycosis. Further efforts are needed to identify the undiagnosed burden of coccidioidomycosis in the border region including the states of Sonora, Arizona, Chihuahua, and New Mexico.

SERA ANTI-Coccidioides spp ANTIBODIES IN DOMESTIC DOGS AND WILD RODENTS IN MEXICO

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Background: In the presence of clinical cases and prevalence of coccidioidomycosis infection $\geq 10\%$ in humans, the Mexican states of Baja California, Sonora, Chihuahua, Coahuila, Nuevo León, Zacatecas, Durango and San Luis Potosi are considered as the main endemic regions for this mycosis in the country. However, the prevalence of coccidioidomycosis infection in domestic and wild mammals in Mexico is virtually unknown.

Objectives: 1) To determine the prevalence of coccidioidomycosis infection in dogs in Torreón (Coahuila), San Luis Potosi, (San Luis Potosi) and Ensenada (Baja California) and prairie dogs from the Chihuahua desert. 2) To compare the results with those obtained in previous similar surveys.

Materials and Methods: Sera from urban dogs and wild rodents was tested employing the Ouchterlony double immuno-diffusion technique, with coccidioidin produced at UNAM as an antigen.

Results: One hundred domestic dog sera were tested, and three (3%) tested positive for anti-Coccidioides antibodies; of 158 sera from prairie dogs, 113 (71.5%) tested positive.

Conclusions: Our current results are similar to previous surveys performed in México that have shown a low prevalence of seropositivity in domestic dogs. Conversely, we found a very high rate of positive sera in wild rodents. To validate the role of serology in domestic dogs it will be necessary to carry out serological testing in suburban domestic dogs, where environmental conditions allow greater exposure to the fungus.

The Impact of Changing Laboratory Reporting and Testing Practices on the Epidemiology of Coccidioidomycosis in Arizona, 2008 - 2013

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Background: Two-thirds of all coccidioidomycosis cases reported nationally reside in Arizona. Between 2009 and 2013, there were significant changes in the number of cases reported annually to the Arizona Department of Health Services (ADHS). Laboratory A, a large commercial laboratory, altered its reporting practices in 2009 and enzyme immunoassay (EIA) testing method in 2012. We investigated the impact of laboratory reporting and testing practices on the epidemiology of reported coccidioidomycosis in Arizona.

Methods: Data from the ADHS surveillance system, hospital discharge database, and vital statistics database were analyzed to assess trends in the incidence of coccidioidomycosis. Coccidioidomycosis-associated hospitalizations were identified using ICD-9 codes.

Results: The incidence of reported coccidioidomycosis increased from 78 (n=4,768) in 2008 to 108.1 (n=7,109) reported cases per 100,000 population in 2013, a 48% increase. The proportion of cases reported by Lab A increased from 28.7% in 2008 to a peak of 75.8% in 2011, followed by a decline to 45.8% in 2013. There were corresponding changes in the age and gender distribution of reported cases. In 2008, 48% of reported cases in Arizona were female. From 2009 – 2012, 56.5% of reported cases were female while in 2013, 49.1% of reported cases were female. A similar trend was not observed among hospitalized cases with a majority of patients being male throughout the analysis period. Cases reported by Lab A had a median age of 46.7 years while cases reported by other labs and healthcare providers had a median age of 51.8 years. However, the age distribution of cases reported by Lab A in 2013 changed significantly with the median age of reported cases increasing from 44 years in 2012 to 50 years in 2013. No significant changes were observed in the median age of hospitalized cases during the analysis period.

Conclusion: Laboratory reporting and testing practices contributed significantly to the observed changes in the epidemiology of coccidioidomycosis from 2008 to 2013. Cases reported by Lab A differed in terms of age and gender from cases reported by other healthcare providers and laboratories. Additional data are needed to understand the changing demographics of reported coccidioidomycosis in Arizona.

The Epidemiology of Coccidioidomycosis in Southern Arizona

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The Arizona Department of Health Services (ADHS) and the University of Arizona College of Epidemiology and Medicine (sections of Pulmonary and Infectious Diseases)

Background: Coccidioidomycosis (Valley fever) is endemic in Arizona. However, it does not occur uniformly in all counties in the state. A varying geographical distribution and incidence in the southern Arizona counties bordering Mexico has been noted in the past, but has not been specifically examined. We decided to explore this incidence gradient in further detail.

Methods: Cases reported from 2006 to 2012 from Cochise, Pima, Pinal, Santa Cruz, and Yuma counties were extracted from the ADHS Medical Electronic Disease Surveillance Intelligence System (MEDSIS). ArcGIS was used to geocode the cases to their home addresses. SAS 9.3 was used for statistical analysis.

Results: In 2012 the incidence of coccidioidomycosis/100,000 persons was highest in Pima and Pinal counties followed by Santa Cruz, Cochise, and Yuma, as shown in the graph. The incidence for each county increased from 2006 to 2012. In addition, 51% of all cases were reported in females compared to 46% for men; in 5%, the gender was not known.

! Conclusions: The gradient in incidence across the southern Arizona counties has not been emphasized or explained. We were able to geocode cases and underscore those differences in incidence across these border counties. Further analysis will focus on potential reasons for this gradient. Unlike other studies, our analysis found more women reported with coccidioidomycosis than men.

Conducting a study on the economic burden of Valley Fever in California: results and lessons learned

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Introduction: California observed an increased incidence of coccidioidomycosis (Valley Fever, FV) in the past decade, increasing the state cost burden. We found only two economic studies - a cost-effectiveness of a potential VF vaccine, and total charges of just VF-associated hospitalizations in California. We estimate the economic burden of VF in California.

Methods: California VF incidence and natural history data were from the California Department of Public Health and other literature. Guidelines and expert interviews determined management and utilization under different VF presentations (primary pneumonia, chronic, and disseminated disease). VF-associated hospitalizations were from California Patient Discharge Dataset (ICD-9 codes 114.0-114.5 and 114.9). Costs were 2012 national estimates- hospitalization from HCUP, outpatient services Medicare national physician fee schedule, and drug costs Red Book®. Direct costs include diagnostic tests, treatment and hospitalization.

Results: There were 4,094 reported VF cases in California in 2012. We estimate that an additional 1,178 VF cases were misclassified as community acquired pneumonia (total of 5,272 people with VF). Diagnosis includes immunodiffusion, titer measurement and chest x-ray. CT scan and lung biopsy were used to diagnose pulmonary nodules, and lumbar puncture for meningitis. Mean annual diagnosis cost is \$2,507/person with VF. Treatment mainly included fluconazole ($\geq 90\%$), and itraconazole, voriconazole and amphotericin B for the remainder, costing a mean of \$3,898/person with VF annually. Primary pneumonia has the highest diagnosis (\$273) and treatment costs (\$3,031 annually) across everyone with VF. In 2012, there are 3,361 hospitalizations with principal diagnosis of VF, costing a total \$87,376,261 (\$25,997/person hospitalized).

Conclusions: Diagnosis and treatment of VF is costly and incurs significant economic burden on California and VF patients and their families. Diagnosis can be unexpectedly costly. Oral fluconazole costs are reasonable (\$24/day) but intravenous amphotericin B can cost as high as \$625/day, though its use is rare.

Whole Blood Cytokine Patterns after *in vitro* Antigen Stimulation among Patients with Various Types of Coccidioidomycosis

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The Southern Arizona Veterans Affairs Medical Center (SAVAHCS), the University of Arizona, and the University of California at Davis.

Background: Cellular immunity is an important factor in the control of human coccidioidal infection. The cytokine pattern expressed after *in vitro* incubation of whole blood with coccidioidal antigens is reflective of this response but remains incompletely defined. In particular, the expression of pro-inflammatory cytokines such as IL-6 and IL-17 has not been reported in human coccidioidomycosis.

Methods: Patients seen at the Coccidioidomycosis Clinic at SAVAHCS were studied. Heparinized whole blood was obtained by venipuncture and incubated with 20 µg/mL of the coccidioidal antigen preparation T27K for 18 hr and supernatant collected. Cytokine concentrations were determined by flow cytometric bead assay or, in the case of IL-17, by a high-sensitivity ELISA.

Results: A total of 72 subjects were studied including 26 with primary pulmonary coccidioidomycosis, 11 with nodules or cavities, 4 with chronic pulmonary disease, and 19 with extrathoracic dissemination. In addition, 12 healthy controls, 6 of whom were immunologically responsive and 6 of whom were not, were assessed. Median cytokine concentrations (pg/mL) for each group are shown in the table below.

Clinical Group	IL-2	IFN-γ	IL-4	IL-10	TNF-α	IL-6	IL-17
Healthy immune (6)	315	194	0	0	41	691	0.8
Healthy non-immune (6)	0	4	0	0	0	54	0.04
Pulmonary disease (41)	795	198	0	0	17	1371	5.7*
Disseminated (19)	516	101	0	0	327	3705	6.9*
	*p<0.01 compared to healthy immune donors						

Conclusions: Whole blood incubated with T27K produces a significant increase in the early pro-inflammatory cytokine IL-17 among patients with clinically active coccidioidomycosis compared to those with resolved, asymptomatic infection. Trends were also seen with IL-6 and TNF-α. Assays for these cytokines could serve as markers for active coccidioidomycosis.

2014_ IL-8 Receptor and Resistance to *C. immitis* Infection Trends Were also Seen with IL-6 and TNF- α . Assays for These Cytokines Could Serve as Markers for Active Coccidioidomycosis.

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VA Healthcare San Diego and Division of Infectious Diseases at UCSD School of Medicine

Background: The pathological response in coccidioidomycosis is a pyogenic granuloma. That is, within the granulomas that are composed of macrophages and lymphocytes there are collections of neutrophils (PMN). Relatively few infections generate that kind of granulomas. In coccidioidomycosis ruptured spherules appear to be chemotactic for PMN as one sees PMN entering those structures and adjacent to the open end of the fungus. However, the chemokine and the PMN receptor is unknown so we tested the hypothesis that ELR⁺ CXC chemokines are involved in this response by infecting IL-8r KO mice. Mice do not have a gene for IL-8 but they have three chemokines (MIP-2, KC, and Lix) that bind to the mouse homologue of the IL-8r, which is encoded by Cxcr2 on chromosome 1.

Methods: We purchased IL-8r KO breeding pairs on a BALB/c (B/c) background from Jackson Labs. By keeping them on oral Septra in their drinking water we could breed them as homozygous KO mice. We infected IL-8r and B/c.D2 Nramp1 congenic mice as controls intra-nasally. We necropsied mice 14 days later for quantitative mycology, and we did a BAL on 4 mice/group to measure cytokines and to assess cell numbers, and the number of L6g/CD11bhi cells (PMN) in the BAL. The IL-8rKO mice had only half the number of PMN in the BAL. There was also a marked difference in the BAL cytokines; IL-8 KO mice made 5-10x as much IL-17A and MIP-2, and more IFN γ . They did not make more IL-1 β , TGF β , IL-6, or IL-23, so the impetus for IL-17A production is not known. Uninfected mice did not have higher levels of IL-17A in the BAL. Most importantly, the IL-8r KO mice had only 1/10th the number of CFU in their lungs.

Conclusions: We conclude that IL-8r has a negative effect on resistance to coccidioidomycosis that dampens the TH17 and TH1 immune responses. We do not know if this is a direct or an indirect result of PMN in tissues.

A Survey of Lectin Reactivity to *Coccidioides* in Infected Human Lung Tissue

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Introduction: Coccidioidomycosis (Valley Fever) is caused by inhalation of arthrospores from the soil-dwelling fungi, *Coccidioides immitis* and *posadasii* followed by spherulation in the lung. We hypothesized that coccidioidal glycosylation patterns may be distinct from mammalian glycosylation and bind differentially to a panel of lectins.

Methods: Lectin-based immunohistochemistry was performed with 20 different biotinylated lectins using formalin-fixed paraffin-embedded human lung tissue from patients with coccidioidomycosis. Subsequently, lectin affinity chromatography was employed to purify fungal glycoproteins from a *Coccidioides* lysate and subjected to mass spectrometry analysis.

Results: From a survey of 20 lectins, *Griffonia simplicifolia* lectin II (GSL II) and succinylated Wheat Germ Agglutinin (sWGA) both bound specifically to endospores and spherules in infected human lung, but did not bind to adjacent lung tissue. Other lectins such as *Phaseolus vulgaris* Erythrolectin (PVE) bound to lung tissue, but not spherules and endospores, while concanavalin A (conA), in particular, bound spherules, endospores and adjacent tissue. GSL II bound strongly to laboratory-grown *Coccidioides* lysate (*Coccidioides* lysate) in an enzyme immunoassay format, while sWGA was weak. Eluates from GSL II affinity chromatography revealed glycoproteins larger than 50 kDa by SDS-PAGE. Mass spectrometry identification of the GSL II affinity-purified bands will be presented.

Conclusions: This is the first report that GSL II and sWGA lectins bind specifically to *Coccidioides* endospores and spherules in infected humans. GlcNAc is a principal component of chitin, but may also be found on terminal glycans in coccidioidal glycoproteins. Further research will identify components of *Coccidioides* that are reactive with lectins GSL II and sWGA for use in antigen detection assays.

Screening spherule cell walls for protective vaccine antigens

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Background: Vaccines prepared from spherule cell walls protect mice from intranasal (i.n.) coccidioidal infection but are complex and locally irritating. We have conducted sequential screening of the proteome of the cell wall fraction. Previously, tandem mass spectrometry identified 650 gene products associated with an in vitro-grown spherule cell wall fraction. From these, 1715 exons >40 bp in size were successfully expressed in vitro. By ELISA, 134 demonstrated significant ($p < .01$) reactivity to patient sera (CF 1:16 – 1:256; $n=65$) as compared to sera from naïve persons ($n=24$). *E. coli*-expressed seroreactive ($n=132$) plus nonseroreactive ($n=51$) peptides were placed in two 96-well plates.

Methods: Peptide pools were prepared as 8 row-wise and 12 column-wise from both plates (40 pools total). Each pool was added to splenocytes from mice that had been immunized with either spherule cell walls or sham vaccination (prime, boost at 2 weeks and splenocytes harvested at 6 weeks). Twenty-four hours later cytokine expression (IFN- γ , IL-17A) was measured by flow cytometry.

Results: Decoding of pool results identified 11 peptides with 2-fold or greater cytokine expression by splenocytes from immunized as compared to unimmunized mice. In a follow-up study, none of the 11 peptides contained measurable endotoxin by limulus lysate assay and, tested individually, 4 peptides showed splenocyte reactivity.

Conclusions-Future Plans: We are currently vaccinating mice with these stimulating peptides to determine if they protect against an i.n. coccidioidal infection more than a vaccine prepared from 4 peptides that were not splenocyte-reactive.

VT-1161, a Novel Fungal CYP51 Inhibitor, Improved Survival in a Murine Model of Coccidioidal Meningitis

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Background: VT-1161, a novel fungal CYP51 inhibitor, is efficacious in murine models of candidiasis, cryptococcosis, and respiratory coccidioidomycosis and is currently in Phase IIa studies. It has low toxicity and a long half-life in rodents, dogs and humans.

Methods: Swiss-Webster mice (8 weeks) were infected intracerebrally with 70-90 arthroconidia of *Coccidioides posadasii*, strain Silveira, producing CNS signs and death 7-9 days post-infection (p.i.). Mice were initially treated orally with VT-1161 (25 mpk or 50 mpk once daily), fluconazole (25 mpk twice daily), or placebo, for 7 days starting 48 hrs p.i. Brain and spinal cord fungal burdens were significantly decreased in VT-1161 groups compared to placebo or fluconazole ($p \leq 0.007$). Ten of 11 spinal cords were negative for fungus in mice treated with 50 mpk VT-1161, however, no brain culture was sterile regardless of treatment. Final VT-1161 plasma concentrations were 26 and 52 $\mu\text{g/ml}$ in the 25 and 50 mpk groups, respectively.

Mice were subsequently treated starting 48 hrs p.i. with VT-1161 (25 mpk once daily), fluconazole (25 mpk twice daily), or placebo, for two weeks, then observed for 4 weeks before sacrifice. Brain and spinal cord fungal burdens were determined quantitatively and lung, liver and spleen cultured in toto to assess dissemination.

Findings: Placebo mice died between days 7-9. Mice treated with fluconazole survived the treatment period, but 10/13 mice died the following week. All mice treated with VT-1161 remained healthy until the middle of the third week following withdrawal of drug, when some mice developed transient CNS signs and some lost weight. Two mice were euthanized due to weight loss, weakness, and dehydration on days 39 and 43 p.i., with *Coccidioides* cultured from lung, liver, and spleen as well as brain and spinal cord. No other VT-1161-treated mice had CNS signs at sacrifice, though all brains were positive for fungus. Four weeks following treatment, mean plasma concentration of VT-1161 was 1.5 $\mu\text{g/ml}$.

Conclusion: mice treated with VT-1161 lived significantly longer than mice treated with fluconazole or placebo ($p < 0.001$) and had reduced brain and spinal cord fungal burdens. These studies show that VT-1161 merits further exploration as a treatment for coccidioidal

Hybridization and Introgression among species of *Coccidioides*

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Background: The genus *Coccidioides* is comprised of two closely related and putatively allopatric species, *Coccidioides immitis* and *C. posadasii*. Recent genomic sequence analysis showed that hybridization has occurred between species, and at least one *C. posadasii* type sequence has introgressed in southern California and Mexico *C. immitis*, but with low frequency into the northern California population. This core introgressed region contains ten genes, and the contribution to virulence and host interactions is currently unknown. We are working to define the function and role of the genes and proteins in this introgressed region to determine their importance in fungal virulence and host interactions. Regardless of whether these genes influence pathogenicity, functional characterization of these genes will significantly expand our knowledge of *Coccidioides* biology, which is greatly needed.

Methods-Results: Currently, we have completed analysis of the expression of these genes *in vivo*, by extracting RNA from lungs of mice infected with *C. posadasii* strain Silveira. Using both real time quantitative PCR and RNAseq, we observed a significant increase in several known and novel transcripts that shed new light on fungal pathogenesis and murine response to infection.

Future Plans: Future work will compare these results with infection models using pure *C. immitis* and *C. immitis* with the *C. posadasii* introgression region.

Differentiating Lung Nodules due to Coccidioidomycosis from Lung Cancer using Clinical and Radiographic Features

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Introduction: Coccidioidomycosis is endemic in the Central Valley of California and can commonly present as a solitary lung nodule. Differentiating Coccidioidomycosis nodules from lung cancer nodules can be difficult and often requires invasive diagnostic tests. Prior studies utilizing chest x-ray suggest that 1/3 of high risk lung nodules in an endemic area are due to Coccidioidomycosis. In 2009 we established a multidisciplinary lung nodule program at an academic teaching hospital to facilitate the diagnosis and management of lung nodules.

Methods: This is a retrospective study of 302 patients with confirmed diagnoses of Coccidioidomycosis or lung cancer seen between December 2010 and May 2013. Patient records were obtained from a multidisciplinary lung nodule clinic and data on pertinent demographics, diagnostic tests, and clinical features were abstracted and analyzed. Statistical analysis was performed using SPSS statistical program.

Results: Of the 302 patients, 110 were confirmed cases of Coccidioidomycosis and 192 were lung cancer. In patients with Coccidioidomycosis 65% were male as contrasted with lung cancer where 49% were male. Patients with Coccidioidomycosis were on average younger than patients with cancer. Forty-nine percent of Coccidioidomycosis patients were ex-smokers or current smokers. By comparison 84% of patients with lung cancer were current or ex-smokers. Patients with Coccidioidomycosis were more likely to report occupational history in agriculture, field work, or in the prison system in California (Table 1). The average nodule size for Coccidioidomycosis was 2.6 cm \pm 1.7 while the average size of nodules in patients with lung cancer was 4.08 cm \pm 2.5.

Conclusions: This study demonstrates that despite the improvements in radiographic imaging of lung nodules, 1/3 of the high risk nodules are still due to Coccidioidomycosis. While there are differences in clinical characteristics and radiographic appearance between Coccidioidomycosis and lung cancer, there is significant overlap in each of the characteristics. None of the criteria are individually sufficiently discriminating to accurately inform clinical decision-making. The future goal of our study is to develop a multivariate tool that will allow clinicians to more accurately predict the probability of Coccidioidomycosis.

Table 1: Characteristics of patients with Coccidioidomycosis vs. lung cancer

		Coccidioidomycosis (n=110)	Lung Cancer (n=192)	P-value
Age		51.65 ±13.63	67.94 ± 11.6	.0001
Size		2.6 cm ± 1.7	4.08 ± 2.5	.000001
Gender (M:F)		72:38	93:99	.004
Occupation	Agriculture/Field Worker	33 (.30, 95% CI: .22-.39)	19 (.10, 95% CI: .06-.14)	.000002
	Construction/Mechanic/Military	13 (.12, 95% CI: .07-.19)	41 (.21, 95% CI: .15-.27)	
	Prison Worker	12(.11, 95% CI: .06-.17)	1 (.005, 95%CI:.002-.025)	
	Other	52 (.47, 95% CI: .38-.56)	132 (.69, 95% CI: .61-.75)	
Border	Lobulated	35 (.32, 95% CI: .25-.40)	74 (.39, 95% CI: .31-.45)	<.0000001
	Smooth	34 (.31, 95% CI: .22-.40)	10 (.05, 95% CI: .03-.09)	
	Spiculated	41 (.38, 95% CI: .28-.46)	108 (.56, 95% CI: .49-.63)	
Family History	Hx of Lung Cancer	12 (.11, 95% CI: .06-.17)	37 (.19, 95% CI: .14-.25)	.06
	No Hx of Lung Cancer	98 (.89, 95% CI: .82-.94)	155 (.81, 95% CI: .74-.85)	
Smoking History	Non-smoker	56 (.51, 95% CI: .41-.60)	32 (.16, 95% CI: .12-.22)	<.0000001
	Ex-Smoker	35 (.32, 95% CI: .23-.41)	80 (.42, 95% CI: .34-.48)	
	Current Smoker	19 (.17, 95% CI: .10-.25)	80 (.42, 95% CI: .34-.48)	

Serum Procalcitonin Levels in Acute Coccidioidomycosis Infections

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Rationale: The serum procalcitonin (PCT) assay is a promising biomarker that can reliably identify respiratory infections caused by bacteria. PCT is elevated in bacterial infections, but is normal in viral infections. Coccidioidomycosis (CM) is a fungal infection that most commonly presents as an acute respiratory infection. In endemic regions, primary coccidioidal pneumonia may account for up to 29% of all community-acquired pneumonia (CAP). Symptoms of CM are often indistinguishable from bacterial and/or viral respiratory illnesses. The association of serum PCT levels in CM has never been reported. We performed a prospective study looking at the association between serum PCT levels and acute pulmonary CM.

Methods: This is a prospective pilot study in which patients with symptomatic, newly diagnosed, confirmed or highly probable CM infections were studied. The study cohort fulfilled the EORTC diagnostic criteria for confirmed or highly probable invasive fungal infections. Demographic information, symptoms at the time of phlebotomy, serologic and microbiological data, radiographic results, antifungal and corticosteroid treatments at the time of phlebotomy, history of empiric antimicrobial therapy prior to the diagnosis of CM, and serum PCT levels were collected and analyzed. A nonbacterial range serum PCT cut off of <0.25 $\mu\text{g/L}$ was used.

Results: Twenty patients met study inclusion criteria. Three patients had proven CM and 17 had highly probable infections. The median duration between the onset of symptoms to phlebotomy was 33 days. Nineteen of 20 patients (95%) enrolled had PCT levels <0.25 $\mu\text{g/L}$ (range 0.05 – 0.87 $\mu\text{g/L}$, median 0.05 $\mu\text{g/L}$, and interquartile range 0.05 – 0.05). The only patient with a PCT >0.25 $\mu\text{g/L}$ had a level of 0.87 $\mu\text{g/L}$. This patient was on antifungal therapy. Prior to the diagnosis of CM, 17 (85%) had a presumptive diagnosis of CAP or an unspecified respiratory infection and was later diagnosed as acute pulmonary CM. Fourteen patients (70%) were treated with at least one course of empiric antibacterial therapy before the diagnosis of CM was made.

Conclusion: In this small prospective study, serum PCT levels appear to be in the nonbacterial range in acute pulmonary CM.

A Multi-center Laboratory Investigation of Coccidioidomycosis Enzyme Immunoassay Reproducibility in Patients with Confirmed Disease and Controls

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Background: Coccidioidomycosis or Valley Fever (VF) is a respiratory fungal infection endemic to the southwestern United States (US) with rising reported incidence rates over the last decade causing public health concern. Arizona VF surveillance is based predominantly on laboratory testing, including enzyme immunoassay (EIA), making VF EIA reproducibility and validity critical for determining disease burden. To evaluate the laboratory reproducibility of VF EIA results, we compared EIA IgM and IgG results from two different manufacturers [Immuno-Mycologics, Inc. – Premier *Coccidioides* EIA (Immy) and Meridian Biosciences, Inc. – *Coccidioides* Antibody EIA (Meridian)] using sera from the same patients divided among three laboratories.

Methods: Serum samples from 150 patients with laboratory (immunodiffusion and/or complement fixation) and clinical evidence of VF and 50 de-identified serum specimens (controls) from healthy individuals without travel to endemic areas, (presumed negative for VF), were blinded and distributed frozen to three laboratories after retrospective selection by the Kern County Department of Public Health. Results were analyzed for concordance and percent agreement as a primary outcome. EIA sensitivity and specificity were calculated as secondary outcomes.

Results: Percent agreement for EIA IgM and IgG combined among the three labs was 85.5% for Immy (90% for IgM and 89% for IgG) and 70.5% for Meridian (67% for IgM and 81%, for IgG alone). Of note, Meridian IgM EIA results were positive for 13 of 50 controls in one laboratory. Sensitivity for EIA IgM and IgG combined was 68.5% for Immy and 72.4% for Meridian; specificity was 99.3% for Immy and 91.3% Meridian.

Conclusion: Percent agreement of EIA IgM and IgG among laboratories varies depending on the brand of EIA test kit used. One laboratory appears to have an increase in false positive EIA IgM results with the Meridian EIA test kit, which might explain reports of decreased specificity associated with the Meridian EIA IgM test kit described in the literature. Further studies looking at differences between laboratory methods for both test kits at different laboratories, including the wash step, are needed to determine the etiology of the discordant results. 1926 (max 1950)

Incidence and severity of coccidioidomycosis in subjects receiving corticosteroids, DMARDs or anti-TNF- α therapy

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Background: The risk of symptomatic coccidioidomycosis is increased by therapies that suppress the cellular immune response. Medications that may increase the likelihood of severe coccidioidomycosis include corticosteroids, tumor necrosis factor- α (TNF- α) inhibitors, and disease modifying rheumatic drugs (DMARDs). The primary outcome of this study was to assess if subjects receiving corticosteroid therapy with or without DMARDs and/or TNF- α inhibitors (corticosteroid group) would have an increased risk of symptomatic coccidioidomycosis compared to those on DMARDs and/or TNF- α inhibitors only (non-corticosteroid group).

Methods: Outcomes of subjects aged 18-89 with a diagnosis of symptomatic coccidioidomycosis based on ICD-9 code or a positive coccidioidal serology between January 1, 2010 to July 31, 2013 at SAVAHCS were identified. Subjects also had to be receiving one or more of the following agents for ≥ 2 weeks prior to diagnosis: a corticosteroid, a TNF- α inhibitor, or a DMARD. Subjects with a diagnosis of HIV infection, pregnancy, or malignancy were excluded. Severity of infection was assessed using a scale of mild (not prescribed antifungal therapy); moderate (antifungal therapy given); and severe (extrathoracic dissemination or hospitalization). **Results.** One hundred thirty-eight cases of coccidioidomycosis were identified of which 15 met the study inclusion criteria. Of these patients, the majority were receiving immunosuppressive agents due to rheumatologic/dermatologic conditions. Of these cases, seven were in the corticosteroid group. Four of these patients were on corticosteroid therapy alone. Four of the 7 severe cases were in the corticosteroid group while five of 8 of the mild to moderate cases were in the non-corticosteroid group ($P=0.07$). There was a trend for subjects in the corticosteroid group to receive a median shorter duration of immunosuppressive therapy prior to diagnosis compared to those in the non-corticosteroid group (46 vs 445 d; $P=0.08$).

Conclusions: There was a trend toward more severe coccidioidal disease in those receiving corticosteroid therapy alone or in combination with anti-TNF- α and DMARD, and this occurred with trend toward a shorter duration of medication use.

Annual Meetings of the
Coccidioidomycosis Study Group

No.	Date	Location	Held in Conjunction with
1	July 18, 1956	San Francisco, CA	-
2	December 5-6, 1957	Los Angeles, CA	-
3	December 4-5, 1958	Los Angeles, CA	-
4	December 3-4, 1959	Los Angeles, CA	-
5	December 8-9, 1960	Los Angeles, CA	-
6	November 30- December 1, 1961	Los Angeles, CA	-
7	November 29-30, 1962	Los Angeles, CA	-
8	December 5-6, 1963	Los Angeles, CA	-
9	December 10-11, 1964	Los Angeles, CA	CA Thoracic Society
10	December 7, 1965	Phoenix, AZ	2 nd Cocci Centennial Conference
11	April 19, 1967	Palm Springs, CA	CA Thoracic Society
12	May 1, 1968	Fresno, CA	CA Thoracic Society
13	April 15, 1969	San Diego, CA	CA Thoracic Society
14	April 1, 1970	San Francisco, CA	CA Thoracic Society
15	April 6, 1973	Newport Beach, CA	CA Thoracic Society
16	April 5, 1974	Sacramento, CA	CA Thoracic Society
17	September 30, 1974	San Francisco, CA	Cocci Cooperative Treatment Group
18	April 2, 1975	San Diego, CA	CA Thoracic Society
19	July 31, 1975	San Diego, CA	Cocci Cooperative Treatment Group
20	January 14-15, 1976	San Diego, CA	Cocci Cooperative Treatment Group
21	April 7, 1976	Palo Alto, CA	CA Thoracic Society
22	May 18, 1977	San Francisco, CA	Am Lung Association
23	April 5, 1978	Beverly Hills, CA	CA Thoracic Society
24	May 15, 1979	Las Vegas, NV	Am Lung Association

No.	Date	Location	Held in Conjunction with
25	April 11, 1980	Sacramento, CA	CA Thoracic Society
26	March 28, 1981	San Francisco, CA	CA Thoracic Society
27	May 15, 1982	Los Angeles, CA	AM Lung Association
28	March 20, 1983	La Jolla, CA	CA Thoracic Society
29	March 14-17, 1984	San Diego, CA	4 th Cocci Centennial Conference
30	March 8, 1986	Santa Barbara, CA	-
31	April 4, 1987	Los Angeles, CA	-
32	April 9, 1988	Los Angeles, CA	-
33	April 8, 1989	San Jose, CA	-
34	April 7, 1990	Berkeley, CA	-
35	April 6, 1991	Tucson, AZ	-
36	April 4, 1992	Fresno, CA	-
37	April 3, 1993	Tucson, AZ	-
38	August 24-27, 1994	Stanford, CA	5 th Cocci Centennial Conference
39	April 1, 1995	Bakersfield, CA	-
40	March 30, 1996	Scottsdale, AZ	-
41	March 5, 1997	San Diego, CA	-
42	April 4, 1998	Visalia, CA	-
43	March 20, 1999	Tijuana, BC, Mexico	-
44	April 1, 2000	Berkeley, CA	-
45	March 31, 2001	Tucson, AZ	-
46	April 6, 2002	Davis, CA	-
47	April 3, 2003	Scottsdale, AZ	-
48	April 31, 2004	Rosarito Beach, Mexico	-
49	April 2, 2005	Bass Lake, CA	-
50	April 23-26, 2006	Stanford, CA	6 th International Symposium on Cocci
51	March 29, 2007	Tempe, AZ	-

No.	Date	Location	Held in Conjunction with
52	April 5, 2008	San Diego, CA	-
53	April 4, 2009	Bakersfield, CA	-
54	March 27, 2010	Surprise, AZ	-
55	April 2, 2011	Davis, CA	-
56	March 24, 2012	Tucson, AZ	-
57	April 6, 2013	Pasadena, CA	-
58	April 5, 2014	Phoenix, AZ	-