PROCEEDINGS OF THE 57TH ANNUAL

COCCIDIOIDOMYCOSIS STUDY GROUP MEETING

April 6, 2013

Pasadena, California

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Meeting Number 57

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Sheraton Hotel

Pasadena, California

Antonino Catanzaro, M.D.

Coccidioidomycosis Study Group Chairperson

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Meeting Agenda

7:00 A.M. **Breakfast and Registration**

8:00 A.M. **Opening of Meeting and Introductions**

8:15 - 9:45 A.M. **Ecology and Epidemiology**

Moderator: Rebecca Sunenshine

- Coccidioidomycosis in Younger Populations, Arizona, 2011.
  Stephanie McDougall and Clarisse A. Tsang
- The Acquisition of Coccidioidomycosis among Employees at Mayo Clinic Arizona during a Major Construction Project.
  D. Lake, Y. Ruiz, S. Duffy, T. Pitta, M. Clarkson and J.E. Blair
- Epidemiology of Coccidioidomycosis in Maricopa County, AZ: Hospitalizations, Emergency Room Visits and Deaths – 2006-2011.
  John Keenan, Andrean Bunko Patterson, John Galgiani, Lisa Higgins, Susan Hoover and Rebecca Sunenshine
- The Binational Project Improving the Epidemiology of Coccidioidomycosis in the Border Region of "Four Corners" Arizona-Sonora and New Mexico-Chihuahua.
  O. McCotter, P. Dulin, R. Guerrero, G. Barrios et al.
- The Epidemiology of Coccidioidomycosis in Los Angeles County, California – 1973-2011.
  Ramon E. Guevara, Tasneem Motala and Dawn Terashita

9:45-10:00 A.M. **Coffee Break**

10:0-11:30 A.M. **Laboratory and Experimental Science**

Moderator: David Engelthaler

- Dectin-2 Is Not Required for Resistance to Coccidioidomycosis in Mice.
  Joshua Fierer, Shinobu Saijo and Suganya Viriyakosol
- Histopathology of the Avirulent Coccidioides Mutant CPS-1 in Immunocompetent and Immunodeficient Mice.
  Lisa F. Shubitz, Hien T. Trinh, Marc Orbach, Sharon M. Dial and John N. Galgiani
- Searching for Microbial Antagonists to Coccidioides immitis, the Valley Fever Fungus
  Joe Baal, Gerry Guibert and Antje Lauer
- Measurement of Cell Mediated Immunity Using Whole Blood Flow Cytometry among Subjects with Pulmonary Coccidioidomycosis.
  N.M. Ampel, L.A. Nesbit, S.M. Johnson and D. Pappagianis
- Whole Genome Population Sequencing of *Coccidioides*.
  David M. Engelthaler, Chandler Rope, Elizabeth M. Driebe, James M. Schupp, John Gillece, George R. Thompson, Bridget Barker and Paul Keim

- Molecular identification of *Coccidioides* spp. haplotype in histopathological samples
  C.G. Gonzalez-Becuar, R. Laniado-Laborin, L.R. Castanon-Olivares, A.A. Areola-Cruz, J.A. Luna-Isaac, J. Cordova-Guerrero and R. Muniz-Salazar

11:30 A.M.-12:00 P.M. **Remembrance of Hans Einstein**
by Royce Johnson

12:00-12:45 P.M. **Lunch**

12:45-1:30 P.M. **Business Meeting**
Chairperson: Antonino Catanzaro

1:30-3:15 P.M. **Clinical Science**
Moderator: Janis Blair

- Comparison of Enzyme Immunoassay to Immunodiffusion and Complement Fixation for Coccidioidomycosis Diagnosis and Surveillance.
  R. Lusk, N. Tetein, R. Sunenshine and L. Erhart
- The Impact of Early and Short-Term Use of Corticosteroids on the Clinical Course of Patients with Primary Pulmonary Coccidioidomycosis: a Retrospective Case Control Study.
  N. Azadeh, Y.-H.H. Chang, S. Kusne, et al.
- Characteristics of Symptomatic Coccidioidomycosis that Correlate with Provision of Antifungal Treatment and Relationship to Clinical Outcomes.
  N. Mendoza, B. Coakley, Q. Wu and J.E. Blair
- Seroprevalence of Coccidioides spp., Infection in Dogs in Mexico.
  L.R. Castanon-Olivares, L.A. Alvarez, C Segundo-Zaragoza, et al.
- Dissemination of Coccidioidomycosis in a Patient with S>E. Experience with Four Pregnancies.
  N. Blattman and T. Kuberski.
- Clinical Evidence for Coccidioidomycosis and Etiology for Sarcoidosis.
  I. Yourison and T. Kuberski.
- Abortion and Disseminated Infection by *Coccidioides posadasii* in and Alpaca Fetus.
  F.A. Uzal, S.S. Diab, J.P. Garcia, S.M Johnson, L. Erin, E.L. Carlson, D. Pappagianis and J. Smith

3:45-4:00 P.M. **Coffee Break**

4:00-5:00 P.M. **Unusual Cases of Coccidioidomycosis**
Moderator: Rafael Laniado-Laborin

- First Report of Splenic Abscesses Due to Coccidioidomycosis.
  S. Assar, S. Chhaya and T. Kuberski
- Monoarticular Coccidioidal Synovitis in a Pediatric Patient.
  D. Dimitrova, W. Mason, L. Ross and B. Shaham

- Severe Granulomatous Co-infections in a Child.
  Susanna Felsenstein, Jill Hoffman and L. Ross
- Coccidioidomycosis and Non-tuberculous Mycobacteria: You Can Have Ticks and Fleas. N. Liang

7:00 P.M. **Dinner/Adjournment**

Coccidioidomycosis in Younger Populations, Arizona, 2011

Stephanie McDougall, MPH and Clarisse A. Tsang, MPH

Arizona Department of Health Services

Background

Coccidioidomycosis (Valley Fever) is an emerging fungal disease endemic to the southwestern United States, and parts of Central and South America. Coccidioidomycosis became laboratory reportable in Arizona in 1997, and enhanced surveillance of reported coccidioidomycosis cases was conducted from 2007-2008. Since implementation of the mandatory laboratory reporting requirement, reports of coccidioidomycosis have drastically increased in Arizona. In 2008, Arizona received 4,768 reports of coccidioidomycosis. A major laboratory changed its reporting practices by beginning to report positive coccidioidomycosis enzyme immunoassay results without confirmation, which increased Arizona's numbers to 10,233 cases in 2009. In 2011, Arizona's numbers increased to 16,472 cases. In 2011, the Arizona Department of Health Services (ADHS) began an investigation to better understand the impact of coccidioidomycosis in younger populations.

Methods

Cases reported to ADHS from January through December 2011 that were 25 years old or younger at the time of diagnosis were contacted by telephone and interviewed with a standardized questionnaire. Data was analyzed using SAS software.

Results

Data from 294 patients that were ≤25 years old from 2011 were included in the analysis. The mean age was 16 years and 54% of cases were female. Whites made up the majority of cases (76%), followed by blacks (5%), Asians (5%), and Native Americans (2%). Hispanics of all races comprised 20% of cases. Asthma (17%) was the most common comorbidity whereas diabetes was only 2%. The most common symptoms were fatigue (77%), fever (60%), cough (58%), headache (52%), shortness of breath (49%) and sore throat (44%); 51% of cases experienced 3 or more symptoms. Symptoms lasted a mean of 76 days. The mean length of time to seek care for symptoms was 42 days, and it took a mean of 3 visits before a case was tested for coccidioidomycosis. Antifungals were prescribed for only 35% of cases; fluconazole was the most commonly prescribed treatment (29%).

Conclusions

The findings of this investigation give us insight into the impact of coccidioidomycosis on younger populations. We hope that these findings will guide public health in giving recommendations for patient care and treatment of coccidioidomycosis for this population.

The Acquisition of Coccidioidomycosis among Employees During a Major Construction Project

Authors: D. Lake[1], Y Ruiz[1], S Duffy[1], T Pitta[2], M Clarkson[2], JE Blair[3]
[1]Arizona State University
[2]Division of Research Mayo Clinic Arizona
[3]Division of Infectious Diseases, Mayo Clinic Arizona

Introduction: *Coccidioides* species grows in the desert soil, and infects humans and animals following the inhalation of airborne fungal spores (arthroconidia). Arthroconidia become airborne through a number of mechanisms, including disruption of desert soil during construction activities. To minimize the effect of construction on the presence of airborne arthroconidia, recommendations for the control of construction-related dust have been written and codified by municipalities in the endemic area; however, the efficacy of such measures has never been tested. In February 2012, our institution began a building project which required excavation of a large amount of native desert soil. This construction was adjacent to a hospital and outpatient building on the medical campus.
Purpose: We sought to compare the 1 year acquisition of CM in employees working adjacent to the construction, with the acquisition in employees working at another campus 15 miles away.
Methods: Immunocompetent subjects without a history of clinical CM, or a prior positive skin test or serology were recruited from 2 healthcare campuses in Maricopa County. A questionnaire was administered and blood drawn. 0.5cc whole blood was incubated with formalin-fixed Coccidioidin (CDN-F) for 24 hours at 37°C. After incubation, red cells were lysed and the remaining lymphocytes were stained with anti-CD3 and anti-CD69 antibodies. Flow cytometry assessed activated CD69-positive lymphocytes from the CD3 population. One year later, the assay was repeated in employees who were previously assay-negative, to ascertain whether any new coccidioidal infection had occurred in the interval. This study was approved by the Mayo Clinic Institutional Review Board.
Results: 329 employees were enrolled. Initial testing indicated that 22 of 184 (12%) from Campus A and 19 of 145 (13%) from Campus B were positive for prior coccidioidal infection, a difference that was not statistically significant (95% CI 9.2 – 16.6, p=.87). Negative subjects continued in the study, and 1 year later, 2/129 (1.5%) from Campus A and 11/99 (11%) from Campus B were positive, p<.002. The campus with the construction did not have an increased rate of coccidioidal acquisition.
Conclusion: Though the rate of coccidioidal acquisition was significantly different between campuses, it was not higher on the campus with construction. Further study to investigate potential differences between the campuses is ongoing.

Epidemiology of Coccidioidomycosis In Maricopa County, AZ: Hospitalizations, Emergency Room Visits and Deaths – 2006-2011

Authors: John Keenan, MSPH, Andrean Bunko-Patterson, MPH, Rebecca Sunenshine, MD

BACKGROUND

Over 70% of Arizona coccidioidomycosis cases occur in Maricopa County (MC). Both Arizona and MC have seen increased reported coccidioidomycosis rates over the past decade, however reporting changes in 2008 led to challenges in interpreting disease burden and trends. We characterized MC coccidioidomycosis morbidity and mortality from 2006 to 2011 taking reporting changes and age into account to determine actual disease trends.

METHODS

Three data sources were used to describe MC coccidioidomycosis epidemiology from 2006-2011; reportable disease surveillance, emergency department (ED) visits and hospitalizations from de-duplicated hospital discharge records (HDD), and the death certificate record database. We calculated crude rates, rate ratios (RR), and 95% CI as well as age-adjusted rates utilizing weights from the 2000 US standard population for all data sources. Linear regression was used to test for trend over multiple years.

RESULTS

Maricopa County Department of Public Health received over 41,000 coccidioidomycosis case reports from 2006-2011. Crude and age-adjusted rates increased significantly from 113.25 to 343.82 per 100,000, and from 116.46 to 346.42 per 100,000respectively ($p<0.01$). Similarly, age-adjusted rates increased from 2009 to 2011 (209 to 346 per 100,000; $p<0.01$), during which no known reporting changes occurred. HDD analysis resulted in 1,489 individuals with ED visits and 9,204 individuals hospitalized with coccidioidomycosis during the study period. Both ED and hospitalization crude and age-adjusted rates increased significantly over time. The 250 coccidioidomycosis-associated deaths identified during 2008-2011 demonstrated increasing crude mortality rates over time, however this dissipated with age-adjustment.

CONCLUSION

Crude and age-adjusted rates of reported coccidioidomycosis cases, ED visits, and hospitalizations have increased significantly since 2006; however, age-adjusted mortality rates have remained stable. Increased rates of coccidioidomycosis reports and hospitalizations suggest an actual increase in disease burden rather than solely due to increased testing. Stable mortality rates may reflect advances in coccidioidomycosis therapy.

The Binational Project improving the Epidemiology of Coccidioidomycosis in the Border Region of "Four Corners" Arizona-Sonora and New Mexico-Chihuahua

O. McCotter [1], P. Dulin [3], R. Guerrero[1], G. Barrios [4], K. Perez-Locket [3], C. Golenko[1], F. Navarro Galvez [2], C. Zapata[4], O. Alba Sergio [2], M. Alicia Bueno [4], C. Smelzer[3], R. Aguayo[2], C. Vera[4] C. Soto[2], G. Carrete[4], K. Komatsu [1], N. Hernandez [2]

[1] Arizona Department of Health Services [2] Servicios de Salud de Sonora [3] New Mexico Department of Health [4]Servicios de Salud de Chihuahua.

Federal Collaborators: C. Contreras[5], J. Harris[6], S. Montiel[7], I. Hernandez [5], B. Park[6], R. Philen[7], R. Florez[5], S. Waterman[7], M. Lindsley[6], M. Fonseca-Ford[7]

BACKGROUND: The United States-Mexico (US-MX) Border region is geographically located in an endemic area for coccidioidomycosis. The clinical presentation of primary coccidioidomycosis (Valley Fever or 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 surveillance system for coccidioidomycosis. The "Four Corners" project was initiated by federal, state, and local collaborators to enhance binational surveillance.

METHODS: The methods required to build and sustain this border binational regional surveillance effort are multifaceted. One of the first efforts was to insure the laboratory capacity to diagnose coccidioidomycosis. In September 2011, a training for coccidioidomycosis diagnostics was held at InDRE (Mexico Federal Reference laboratory - Mexico City, MX) and offered by CDC/Mycotic Diseases Branch. Sonora and Chihuahua have attended the laboratory training as well as CME courses. The information from the CME event was recorded and translated into Spanish as well. Declarations of Cooperation were signed between Arizona and Sonora, and New Mexico and Chihuahua to implement a regional program for epidemiological surveillance of cocci.

RESULTS: The initial steps to advance this effort included establishing laboratory capacity in order for states to have the capacity to detect coccidioidomycosis. In this pilot study the State Laboratories of Sonora and Chihuahua have identified patients testing positive for coccidioidomycosis from previously established surveillance systems for Tuberculosis and Influenza.

CONCLUSION: These ongoing efforts are aimed to further estimate the disease burden of coccidioidomycosis in the border regions including Sonora, Arizona, Chihuahua, and New Mexico. The integration of public health agencies at all levels has strengthened support and collaboration. This effort will help to systematically enhance binational epidemiological surveillance of coccidioidomycosis and provide information for joint analysis in the US-MX Border - specifically in the shared border region of these states.

The Epidemiology of Coccidioidomycosis in Los Angeles County, California, 1973-2011

Authors: Ramon E. Guevara, Tasneem Motala and Dawn Terashita

Background

Between 1973 and 2011, Los Angeles County has seen the annual incidence of coccidioidomycosis rise from less than 50 cases to over 300 cases, with sharp increases starting in 2004.

Methods

We examined electronic passive surveillance data from 1992-2011 to describe demographic, medical history, exposure history, and geographic case characteristics. Coccidioidomycosis is a mandated reportable disease in Los Angeles (LA) County and confirmation of reported cases require both laboratory and clinical diagnosis.

Results/Analysis

Of the 24 health districts of LA County, 19 had increases from 100% up to 1500% between the intervals 2000-2003 and 2008-2011. Most of the cases (68%) resided in the northern part of LA County - namely Antelope Valley, San Fernando Valley, and West Valley health districts. From 1992-2003 the male-to-female ratio of cases increased from 2.1 to 5.7. From 2004-2011, this ratio ranged between 1.4 and 2.2. White and Hispanic cases became much more numerous than Asian and black cases after 2003. These demographic changes might be due to community development and migration of new residents and workers with the construction boom that started in 2003-2004 in the Antelope Valley. Mortality was 9% but measured 13% during years when survival status was missing for 0-4% of cases. Incidence rate increased with age, and adults ≥65 years-old had more cases than any other age group in 2011. Regarding exposures one to four weeks before illness, cases that lived in one of the three "endemic" valley health districts had higher odds of being in an area in sight of construction [odds ratio = 5.5 (95% CI of 3.9-7.7)], being in a dust storm [odds ratio = 3.3 (2.2-4.9)], using outdoor recreational vehicles like dirt bikes [odds ratio = 3.3 (1.2-8.4)], and participating in an outdoor activity involving dirt, like landscaping [odds ratio = 1.7 (1.3-2.3)] than all other LA County resident cases. Cases not residing in one of these "endemic" health districts had higher odds of travelling to non-LA County endemic areas like Arizona and Central California (R=2.0 (1.7-2.5)). Missing data limits our analysis but substantially more cases are being reported throughout LA County since 2008.

Dectin-2 is not required for resistance to coccidioidomycosis in mice

Authors: Joshua Fierer, Shinobu Saijo, and Suganya Viriyakosol

Background

The innate immune response is triggered by host proteins that are encoded in the genome and recognized conserved structures on microbes (pattern recognition receptors). The recognition proteins can be circulating like CRP, complement, and MBL or cellular. The latter can be on surface membranes, in endosomes or cytosolic. C-type lectins are membrane proteins that recognize carbohydrates. Dectin-1 is expressed by myeloid cells and recognizes β-glucans. It is required for resistance to coccidioidomycosis in mice (Viriyakasol et al. mBio, in press). Dectin-2 is expressed on myeloid cells, it recognizes high mannose structures, and it is involved in the immune response to C. albicans infections.

Methods/Results

We stimulated peritoneal macrophages or bone marrow derived dendritic cells DC with either formalin-killed spherules (FKS) or A-FKS (FKS that was boiled in NaOH to remove mannose residues). Macrophages from Dectin-2 knockout (KO) mice made less TNFα, IL-6, and MIP-2 than did control macrophages. DC from Dectin-2 KO mice made less IL-10 but the same amount of TNFα, IL-6, GMCSF, and IL-1β as control DC. Surprisingly, they responded similarly to FKS and A-FKS. Finally, we infected mice intra-nasally with C. immitis RS, and they were not more susceptible to coccidioidomycosis as measured by lung and spleen CFU 14 days post infection.

Histopathology of the Avirulent *Coccidioides* Mutant, CPS1, in Immunocompetent and Immunodeficient Mice

Authors: Lisa F. Shubitz, Hien T. Trinh, Marc Orbach, Sharon M. Dial, and John N. Galgiani

Introduction

The delta-CPS1 mutant of *Coccidioides posadasii* has been shown to be avirulent in C57BL/6 mice. However, we have previously been unable to demonstrate any histological presence of the organism or inflammation in mouse lungs on day 11 or day 14 postinfection (p.i.) following intranasal administration of 50-4400 spores.

Methods/Results

We infected immunodeficient NOD-SCID (NSG) mice, which lack NK cells and all lymphocyte lineages, with 1000 spores intranasal and sacrificed them on days 6 and 14 post infection. Mice remained healthy throughout the observation period, and 3/6 mice had <12 0.5-1.0 mm reddish-grey lesions scattered throughout the lungs at necropsy. Lungs of two mice from each time point were placed in formalin; no organisms were seen on step sections through the entire lung (H&E stain). One of two mice grew colonies from day 14 lung cultures. Additional mice (NSG and BALB/c) were infected with 10,000 spores of CPS1 IN and a cocci-specific immunohistochemical stain applied. Spherules in low numbers were observed on day 6 post infection but not day 3. Spherules appeared to be thin-walled and degenerating and were either surrounded by neutrophils (both BALB/c and NSG) or infiltrated by neutrophils. Most appeared not to have endospores. BALB/c mice inoculated with 25 million spores intranasally and sacrificed on days 1, 3, 4, 5, 7, and 10 post infection showed the development of numerous clusters of spherules with a significant suppurative pneumonia on days 3, 4, and 5. Many of the spherules appeared degenerate and thin-walled as described above, but some successfully formed thin-walled endospores. No endospores were seen rupturing out of the spherules, and on day 5, the majority of spherules were either collapsed or filled up by neutrophils. By day 7 the number of spherules observed in lungs had decreased approximately 90%. By day 10 the remaining spherules were small, occasional and entirely encapsulated within mature granulomas.

Searching for microbial antagonists to *Coccidioides immitis*, the Valley Fever fungus

Joe Baal[1], Gerry Guibert[2], and Antje Lauer[1]

[1] California State University Bakersfield, Department of Biology
[2] Monterey County Department of Public Health

Purpose
This project focused on the isolation and identification of bacteria that can inhibit the growth of *C. immitis*, the fungal pathogen that causes Valley Fever in the Southern San Joaquin Valley of California.

Methods
Bacterial isolates were obtained from two sampling sites in May 2011 on a low nutrient R2A medium supplemented with 10 % soil extract. All pure cultures were first tested against the fungus *Uncinocarpus resii*, which is a non-pathogenic close relative to *C. immitis* by performing challenge assays on R2A media plates. All anti-*U. reesii* bacterial isolates were then further challenged against *C. immitis* in a Biosafety 3 lab. Several strong anti-*C. immitis* bacterial isolates were identified by sequencing of a 1400 bp fragment of the 16S rRNA gene and belonged primarily to different *Streptomycetaceae* and *Bacillaceae*.

Results
A natural microbial antagonist to *C. immitis* might be useful in a bioremediation approach to suppress the growth of the pathogen in areas near recreation sites and schools in Kern County, CA, and elsewhere in the Southern San Joaquin Valley where *C. immitis* growths. Because the antagonist will be a member of the natural bacterial community in the soils treated (only its presence will be enhanced), the impact on other members of the microbial community in the soil might be negligible.

Conclusion
Our results might be applicable to other areas with pathogens that cause infectious diseases similar to coccidioidomycosis (Valley Fever), such as blastomycosis and histoplasmosis. These major pulmonary mycoses of humans all reside in the soil.

MEASUREMENT OF CELL MEDIATED IMMUNITY USING WHOLE BLOOD FLOW CYTOMETRY AMONG SUBJECTS WITH PULMONARY COCCIDIOIDOMYCOSIS

Ampel NM; Nesbit LA; Johnson SM; and Pappagianis D.
From SAVAHCS and the University of Arizona, Tucson, AZ and the University of California at Davis, Davis, CA, USA.

BACKGROUND
Cell mediated immunity (CMI) is an important predictor of outcome in human coccidioidomycosis but there is currently no clinically available test to determine this. We have developed a whole blood assay to measure this.

METHODS
Individuals with coccidioidomycosis donated 5 mL of blood in sodium heparin. To each aliquot of 0.5 mL, either nothing (control), 20 μg/mL of T27K, or 1 μg/mL staphylococcal enterotoxin B (SEB) (both final concentrations) were added. After incubation for 18 hr, red blood cells were lysed, and the proportion of T27K-stimulated $CD3^+$ T lymphocytes expressing CD69 minus unstimulated T lymphocytes was determined by flow cytometry ($CD69^+$).

RESULTS
Forty-one subjects with pulmonary coccidioidomycosis were studied. Ten had an underlying rheumatologic condition, 20 had other conditions, and 11 had no underlying disease (healthy). $CD69^+$ was significantly lower in those with any underlying condition (P=0.013), including those with rheumatologic conditions (P=0.004), than in healthy subjects. Immunosuppressive therapy was associated with lower $CD69^+$ (P = 0.04). No difference was observed between those with immunodiffusion complement fixation (IDCF) titers ≥1:8 compared to those with lower titers (P = 0.839) or among subjects who were receiving antifungal therapy compared to those who were not (P = 0.111). Six subjects, all with underlying diseases, had repeated testing over 98 to 252 days. Four subjects had persistently negative $CD69^+$ responses. One had a positive initial response that was maintained. A final patient initially demonstrated a negative $CD69^+$ response that became positive with repeat testing.

CONCLUSION
A rapid whole-blood analysis to determine coccidioidal CMI is feasible. Differences in CMI among subjects with pulmonary coccidioidomycosis were seen and changes over time occurred.

Whole Genome Population Sequencing of Coccidioides

David M Engelthaler[1], Chandler Roe[1], Elizabeth M Driebe[1], James M Schupp[1], John Gillece[1], George R Thompson[2], Bridget Barker[3], Paul Keim[1,4]

[1]Translational Genomics Research Institute, [2]University of California Davis, [3]Montana State University, [4]Northern Arizona University

Background

Population genetics for Coccidioides has typically relied on targeted sequencing or other molecular analysis. Newer technologies and bioinformatic tools are allowing for population level whole genome sequencing in fungi and other eukaryotes.

Methods

We have recently sequenced 55 genomes of diverse C. immitis and C. posadasii isolates and added those to the previously published 25 whole genomes. Through whole genome SNP typing (WGST) we identified over 400,000 shared SNP loci, providing not only great genetic resolution but also allowing for a better understanding of phylogenetic relationships within and between populations.

Conclusion

This analysis provides phylogeographic context that will be useful for molecular epidemiology, ecology and evolutionary studies of Coccidioides.

Molecular identification of *Coccidioides* spp. haplotype in histopathological samples

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BACKGROUD: It has been reported in scientific literature that *Coccidioides immitis* is found in the Central Valley of California, San Diego and Baja California, while *C. posadasii* is found in the desert regions of North America, Mexico, and dispersed areas in South America. However, the current distribution of *C. immitis* and *C. posadasii* in Mexico is not widely known. Formalin-fixed and paraffin-embedded (FFPE) tissue samples represent an immense but mainly untapped resource for molecular analysis. The aim of this study was to establish the predominant *Coccidioides* species in Baja California.

METHODS: A total of 129 FFPE tissue samples with a positive histopathology diagnosis of coccidioidomycosis (CM) were analyzed. The samples were obtained from the collections of the Pathology Laboratory from the Tijuana General Hospital from 1982 through 2010. The DNA genomic was extracted using DNA kit Ultraclean MicrobialMobi. We amplified the *Ag2/PRA* gene in order to determine the species and haplotype.

RESULTS: The amplification of the 342 bp of *Ag2/PRA* gene was successful in 109 samples (84.5%), however only 65 out of 129 samples were successfully amplified and sequenced. Once edited, they were aligned and compared to the sequence of the *C. posadasii* Silvera strain (Accession No. AF013256). Thus, 54 (83%) samples were identified as *C. posadasii*, and 11 (17%) as *C. immitis*. We identified five haplotypes for *C. posadasii* and three for *C. immitis.*

CONCLUSION: *C. posadasii* is the most abundant species detected in Baja California, which is opposite to the results previously reported. In Baja California, immigrants represent 45% of the local population growth. It might be that patients were diagnosed in Baja California but infected in other parts of Mexico. Unfortunately, we do not have enough clinical data to confirm this theory. More detailed clinical records could be helpful to obtain more accurate information about the infections in specific geographical places.

Comparison of enzyme immunoassay to immunodiffusion and complement fixation for coccidioidomycosis diagnosis and surveillance

Rachel Lusk, Nathalie Petein, Rebecca Sunenshine, Laura Erhart

Background: Interpreting discordant results between different assays for coccidioidomycosis continues to be a challenge.

Methods: We examined serological testing used in coccidioidomycosis diagnosis to determine how enzyme immunoassay (EIA) testing compares to complement fixation (CF) and immunodiffusion (IMDF). Results of all coccidioidal tests were obtained from two laboratories for a 13-month (Lab A) and a six-month (Lab B) period. Test sets of one EIA IgM, one EIA IgG and two IMDF or CF assays run from the same specimen were selected for analysis.

Results: 125 (9%) of 1,445 test sets from Lab A and 4,991 (58%) of 8,564 test sets from Lab B were positive for at least one EIA and negative by CF/IMDF. Medical charts for a subset of these patients with discrepant results were reviewed (Lab A: 125 (100%); Lab B: 246 (5%)) to identify additional coccidioidal testing, coccidioidomycosis symptoms, and clinical diagnoses. Subsequent positive non-EIA testing was identified for 31 (25%) of patients reviewed from Lab A and 12 (5%) of 246 patients reviewed from Lab B. Most patients reviewed had symptoms of coccidioidomycosis (Lab A: 98%; Lab B: 84%). Of charts that included a diagnosis, coccidioidomycosis was diagnosed for 100% of Lab A patients and 30% of Lab B patients. Adjusting for patients with subsequent non-EIA tests identified, the sensitivity and positive predictive value of EIA compared to CF/IMDF varied by laboratory (Lab A: 84%, 62%; Lab B: 98%, 27%). The proportion of discrepant test sets with positive IgM EIA and negative IgG EIA also varied by laboratory (Lab A: 42 (34%); Lab B: 169 (69%)).

Conclusions: This study demonstrates that clinical judgment must be used when interpreting the results of these tests, and that subsequent testing of patients with positive EIA results may be useful. Additional studies are needed to determine how best to interpret EIA-positive results for clinicians and public health officials in the absence of additional test results.

The Impact of Early and Short-Term Use of Corticosteroids on the Clinical Course of Patients with Primary Pulmonary Coccidioidomycosis: A Retrospective Case Control Study

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Background: Primary pulmonary coccidioidomycosis manifests as a febrile respiratory syndrome often associated with hypersensitivity symptoms treatable with a short course of palliative corticosteroids. Long-term use of corticosteroids is a known risk factor for severe or disseminated coccidioidal infection but the effects of short-term use are not known.

Methods: A retrospective review was conducted of immunocompetent patients with acute pulmonary coccidioidomycosis who received systemic corticosteroids for symptomatic relief of coccidioidal-related symptoms. An equal number of age- and sex-matched controls were reviewed. Predetermined end points were assessed.

Results: Seventy-four patients met inclusion criteria for the corticosteroid-treated group, and 74 controls were identified. Cumulative corticosteroid doses (calculated as prednisone-equivalent doses) were 10 mg to 3,600 mg (mean, 206 mg; median, 120 mg). Corticosteroids were prescribed most commonly for rash (43/74 [58%] or asthma/wheezing/cough (30/74 [41%]. Coccidioidal-related hospitalization occurred in 19 patients in the corticosteroid group vs. 22 in the control group (P=.58). Coccidioidal-related symptoms resolved within a mean of 19 weeks (median, 8 weeks [range, 2-208 weeks]) in the corticosteroid group vs. a mean of 32.3 weeks (median, 8 weeks [range, 1-1,040 weeks]) in the control group (P=.38). There were no deaths due to coccidioidomycosis in either group. Relapse of symptoms occurred in 12% of both groups (P>.99). Extrapulmonary dissemination occurred in 2.7% vs 4.0% (P>.99) in the corticosteroid and control groups, respectively.

Conclusion: This retrospective case-control study found no adverse effects of short-term corticosteroid therapy for early symptomatic treatment in acute pulmonary coccidioidomycosis.

Characteristics of Symptomatic Coccidioidomycosis that Correlate with Provision of Antifungal Treatment and Relationship to Clinical Outcomes

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Introduction
The outcome of patients with symptomatic coccidioidomycosis varies widely.

Purpose of study
To compare characteristics of patients treated or not treated for symptomatic coccidioidomycosis to determine which clinical traits are associated with a good clinical outcome without treatment.

Methods
Patients reported to Arizona Department of Health Services for coccidioidomycosis or who had an ICD-9 coccidioidomycosis diagnosis code were identified. After excluding patients who were asymptomatic, had initial disseminated infection, or had inadequate treatment data, the clinical courses and outcomes of patients were reviewed. Good outcomes were resolution of illness or an improved clinical course. Poor outcomes were progressive disease, relapse, dissemination, or death as a result of coccidioidal infection.

Results
From January 1, 1999 through December 31, 2003, 322 patients with symptomatic coccidioidomycosis met the study criteria. 303 had baseline treatment data (113 not treated; 190 treated). Untreated patients were less likely to have diabetes, immunosuppression, or require hospitalization. 20 patients were excluded from the outcomes analysis due to inadequate data. Poor outcomes occurred in 47/283 (17%) patients. Untreated patients were more likely to have a good outcome (90% vs. 80%) and were less likely to relapse. Patients without risk factors for severe infection (male, non-white race, diabetes mellitus, immunosuppression, tobacco use, or lung disease) had fewer poor outcomes, although this was not statistically significant.

Conclusion
The majority of untreated patients with symptomatic coccidioidomycosis in this study had a good outcome. Further studies should address whether treatment could be avoided in patients without risk factors for severe infection.

Seroprevalence of *Coccidioides* spp., Infection in dogs in Mexico

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BACKGROUND

The role of animals in coccidioidomycosis ecology is not well established, but it has been reported that dogs are susceptible to infection. A review of the literature for the past 40 years revealed that there is almost no data in Mexico on canine *Coccidioides* spp infection. Several reports concur that serology has a higher sensitivity for the detection of coccidioidomycosis infection in dogs than skin testing. To determine the prevalence of infection in dogs, serum from dogs in Mexico was tested to identify *Coccidioides* antibodies using two different serologic methods.

METHODS

A total of 77 serum samples from 52 dogs from Torreón, Coahuila. (a coccidioidomycosis endemic area) and 25 dogs from México City (non-endemic area) were tested using the double-immunodiffusion and contraimmuno electrophoresis techniques.

RESULTS

Of 77 samples tested, only 1 serum tested positive (from Torreón).

CONCLUSION

The prevalence of positive serology for coccidioidomycosis in dogs was much lower than expected.

DISSEMINATED COCCIDIOIDOMYCOSIS IN A PATIENT WITH SLE, EXPERIENCE WITH FOUR PREGNANCIES

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BACKGROUND

An 18 year old (1997) Hispanic female was diagnosed with systemic lupus erythematosis with renal involvement. She was subsequently treated mainly with steroids and plaquenil. She developed disseminated coccidioidomycosis at the age of 19 (1998) presenting with bony lesions of the spine and paraspinous abscesses. She underwent multiple back surgeries of the cervical, thoracic and lumbar spine with fusions and placement of Harrington rods. She was initially treated with amphotericin B and then placed on oral diflucan. Her cocci CF titers went from 1:256 to 1:16 prior to her first pregnancy.

RESULTS

Her first pregnancy was at age 21 (2001). She was taking 800 mg of Diflucan at 17 weeks when her pregnancy was diagnosed by ultrasound and the Diflucan was stopped. The fetal ultrasound was abnormal particularly demonstrating fetal skeletal abnormalities. The pregnancy was terminated at 20 weeks.

Her second pregnancy was at age 25 (2005) and terminated at 8 weeks at the patient's request.

The third pregnancy was at age 27 (2007). She was given amphotericin B once a week for the course of her pregnancy. Cocci CF titer was less than 1:2 throughout the pregnancy. At 36 weeks she delivered a normal live infant by cesarean section.

The fourth pregnancy was at age 32 (2011). She was on itraconazole which was stopped when she missed a period. Her cocci CF titers were less than 1:2 throughout her pregnancy. She received no antifungal therapy during this pregnancy and delivered a normal infant by cesarean section. A tubal ligation was done.

CONCLUSIONS

The management of coccidioidomycosis in pregnancy is subject to the clinical circumstances.

ADDENDUM

At age 33 (2012) her cocci CF was less than 1:2. She was diagnosed with squamous cell carcinoma of the anal canal and rectum with liver metastasis; she was sent to hospice.

CLINICAL EVIDENCE FOR COCCIDIOIDOMYCOSIS AS AN ETIOLOGY FOR SARCOIDOSIS

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BACKGROUND

At the 2002 Coccidioidomycosis Study Group Meeting four Arizona patients with sarcoidosis and coccidioidomycosis were presented. In that presentation the question was raised which came first, the sarcoidosis or coccidioidomycosis? It was theorized that certain patients diagnosed with sarcoid in Arizona really had coccidioidomycosis and it was predicted that because of the immunosuppression of steroids these sarcoid patients would eventually express coccidioidomycosis.

METHODS

The opportunity to test this hypothesis came in 2002 when a patient was evaluated in Infectious Diseases consultation for postoperative fever. He had undergone a splenectomy because of thrombocytopenia and hypercalcemia. The surgically removed spleen showed non-caseating granulomas. Three pathologists were in agreement that the spleen changes were typical of sarcoidosis. Evaluations for the various causes of a granulomatous process were negative. There was no evidence for coccidioidomycosis by serology or spleen histopathology. The patient was treated with prednisone for complicated sarcoidosis.

RESULTS

Because of the prediction that this Arizona sarcoid patient would eventually develop coccidioidomycosis the patient was followed prospectively for seven years (2009) before he developed culture proven disseminated coccidioidomycosis.

CONCLUSION

A hypothesis was developed to explain why this patient's sarcoidosis was caused by Coccidioides and how it could support an infectious disease cause for sarcoid. Basically, the primary host immune response to Coccidioides infection involves the cell mediated immune system. The prototypical infectious disease which best illustrates the human cell mediated immune response to infection is leprosy. The hypothesis for why sarcoidosis in this patient was caused by Coccidioides equates to the immune response seen in the tuberculoid form of leprosy. Further studies are needed to confirm the conclusion that a heightened cell mediated host immune response explains the potential infectious disease etiology for sarcoidosis.

Abortion and disseminated infection by *Coccidioides posadasii* in an alpaca fetus

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Background

Coccidioidomycosis is a fungal disease caused by either *Coccidioides immitis* or *Coccidioides posadasii*. Congenital *Coccidioides spp.* infections followed by abortion have been rarely described in humans and in a mare, but no congenital infection or abortion has been described in camelids.

Case Description

An aborted 9-month gestation alpaca fetus with the placenta was submitted for necropsy. Multiple organs of the fetus and the placenta presented pyogranulomas. Multifocally, within these pyogranulomas, there were large numbers of round, 60-100 um fungal spherules (sporangia) with a 4-5 um thick refractile and hyaline double wall. Sporangia contained flocculent basophilic to amphophilic material and, rarely, multiple 5-7 µm endospores. Frequently, the spherules were seen within the cytoplasm of giant cells. The spherules stained positive with GMS and PAS. DNA isolated from placenta and lung was evaluated for *Coccidioides spp.* using real-time PCR, which produced a positive result for both tissues. Conventional nested PCR was used to amplify a region of the ribosomal DNA followed by sequencing and comparison with known strains of *C. immitis* and *C. posadasii*. The sequence obtained was most similar to that of *C. posadasii.* Complement fixation for *Coccidioides spp.* on the dam serum was positive; by quantitative immunodiffusion this animal had a titer of 1:256, which is interpreted as an indicator of disseminated coccidioidomycosis. The dam was euthanized and necropsy revealed pyogranulomas in multiple organs. Most of the granulomas in the dam contained only occasional fungal spherules similar to those described in the fetus. PCR on the dam's tissues is under way. Additional abortion work-up performed on the fetus, placenta and dam's serum was non-diagnostic. It is most likely that the infection was transmitted to the fetus from the dam via placental circulation.

Conclusion

This seems to be the first confirmed case of *C. posadasii* infection in animals in California and the first case of congenital coccidioidomycosis in an alpaca.

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	2nd Cocci 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

Annual Meetings of the

Coccidioidomycosis Study Group

No.	Date	Location	Held in Conjunction with
23	April 5, 1978	Beverly Hills, CA	CA Thoracic Society
24	May 15, 1979	Las Vegas, NV	Am Lung Association
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	4th Cocci 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	5th 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	-

Annual Meetings of the

Coccidioidomycosis Study Group

No.	Date	Location	Held in Conjunction with
48	April 31, 2004	Rosarito Beach, Mexico	-
49	April 2, 2005	Bass Lake, CA	-
50	April 23-26, 2006	Stanford, CA	6th International Symposium on Cocci
51	March 29, 2007	Tempe, AZ	-
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	-

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